THE BRITISH JOURNAL OF OPHTHALMOLOGY

INCORPORATING

THE ROYAL LONDON OPHTHALMIC HOSPITAL REPORTS
THE OPHTHALMIC REVIEW AND THE OPHTHALMOSCOPE

INDEX
VOLUMES XXI-XXX
1937—1946

LONDON:
THE BRITISH JOURNAL OF OPHTHALMOLOGY, LTD.,
24-27, Thayer Street, Marylebone, W.1.

INDEX—Vols. XXI - XXX

Original Communications and Clinical, Pathological and Bacteriological Memoranda.

to interpret the notes Search was continued until no other possible sources of information were left, with the result that only 37 of 1036 death certificates (3 6 per cent) were not traced (Table I)

Omitting these 37 certificates about which no clinical data could be obtained, it was found that the clinical diagnosis or autopsy record tallied with that recorded on the certificate in 964 of 999 cases, while in only 35 cases was the clinical diagnosis of another disease made in life and yet the certificate recorded death from carcinoma of the lung (Table I) The possible reasons for these discrepancies are discussed on p 3, while the type of clinical diagnosis is shown in Table II It is not difficult to understand why metastatic lung cancer (accounting for 23 cases) should occasionally be recorded as primary lung cancer, but it might be expected that non-cancerous conditions should not appear in this way

In regard to the accuracy of the clinical diagnosis of cancer of the bronchus, the death certificates were traced back to the clinical notes in 953 cases (Table IV) In half of these, the diagnosis could be accepted as unequivocal (class I) In another 333 cases, the consultant in charge of the case was confident that the diagnosis was correct, leaving 143 cases (15 per cent) where the diagnosis was in considerable doubt It is evident that where the clinical diagnosis was by no means certain (Table IX, class II) autopsy confirmed the condition in a large proportion of cases (Table V)

These findings are similar to those reported by McKenzie (1956) who used a different method of sampling A questionary was sent from the General Register Office to the certifying medical practitioner in respect of every second death ascribed to cancer of the lung or bronchus in January, 1955 From 770 inquiries dispatched 654 replies were received The validity of diagnosis was classified in much the same way as we have done and it was found that the standard of diagnostic technique was high in only 3 per cent had no confirmatory procedure been adopted Of a total of 634 certificates in only 18 cases was the certification of carcinoma of the lung not supported In 8 of these the lung was found not to be carcinomatous and in the remaining 10 the lung condition was secondary to a primary elsewhere It is interesting to note that in the 8 cases wrongly diagnosed as carcinoma the diagnosis was corrected in 4 by a post mortem examination made subsequent to the issue of the death certificate, and in the remaining 4, where necropsy apparently confirmed the diagnosis, subsequent histopathology disproved the macroscopic findings The system obtaining in most hospitals, whereby the death certificate must be completed by the resident doctor before the post mortem examination is performed, leads in this way to a failure to amend the death certificate when the findings of the necropsy are known It is always possible to overcome this difficulty if the resident doctor will initial Box B on the back of the certificate

In addition to estimating the accuracy of the clincial data upon which the death certificates were based, an analysis was made of the mode of death certification of the cases diagnosed at the two main hospitals Omitting the 20 patients known to be still alive, 813 of 879 cases diagnosed in the hospitals had death certificates which recorded the clinical diagnosis (8 of these were certified in another borough) and there were 66 in which cancer of the lung did not appear on the death certificate (7 5 per cent), or where the disease was not assigned to the coding carcinoma of the bronchus In regard to the cases certified correctly, the clinical diagnosis was unequivocal in 60 per cent and highly probable in another 39 per cent (Table

IX) In the 66 cases certified as dying from other causes, the discrepancy was due either to the method of wording of the certificate or to difficulties in coding (see p 8) Some of these discrepancies could have been avoided if the rules for certification and coding had been perfectly applied But death certification does not and cannot be expected to give an exact picture of morbidity, as a few cases of cancer of the bronchus will be cured and others will die of independent causes, such as accidents and acute diseases

An analysis of the clinical diagnosis in the 294 cases certified as dying of cancer of the lung and submitted to autopsy (Table V) is interesting Where the autopsy was confirmatory of the clinical diagnosis, by far the largest group (76 per cent) had been diagnosed by ancillary methods only In many of these cases there would either not be time to make a firm diagnosis or the consultant would consider that the evidence was strong, even in the absence of histological examination Of the autopsies which revealed the condition for the first time, in one half another condition, either malignant or non-malignant, had been diagnosed and in the remaining half either no attempt at diagnosis had been made or the death was sudden Of the 27 cases in which autopsy did not reveal cancer of the lung, but the death certificate recorded the condition, the diagnosis had been considered to be well established in 9 but in 17 there was no written clinical evidence for it and there was a real error in certification This represents a false positive error in the death certificates of 9 per cent as revealed by autopsy, and this is largely contributed to by the 17 cases for which there was no written clinical evidence that the disease had ever been diagnosed in life When the autopsy records of all the clinical cases in the two hospitals are analysed by Willis's method (Table IV) false positive diagnoses amount to 8 per cent

The conclusion is drawn from all the above facts that the positive error in certification of cancer of the lung in Leeds is small (3 5 per cent) In fact, a larger number of cases which are diagnosed clinically fail to appear among the death certificates (7 5 per cent) The reasons for the errors are partly due to ambiguity of wording of the certificates, partly to mis-diagnosis and partly to the intervention of other causes of death

It had been thought previously (Bonser and Thomas, 1955) that the discrepancy in certification was greater in females than in males and it was suggested that it was possible that more female deaths from metastatic lung cancer were recorded as primary lung cancer than were male deaths from this cause No support could be obtained for this suggestion from this survey, 7 of 10 females and 16 of 25 males having been certified as dying from primary lung cancer when the disease was really metastatic (Table II) It was noted, however, that the female to male sex ratio was lower both for those cases incorrectly certified as lung cancer and for those positively diagnosed cases which were not certified as lung cancer These effects tend to cancel one another out and there is no reason to suppose that the true sex ratio is materially different from that revealed by death certification

CONCLUSIONS

1 1036 death certificates recorded in Leeds city in the years 1950–54 and coded as cancer of the trachea, pleura, lungs or bronchi (*i e* code no 162 and 163) were scrutinised The clinical records of all but 37 were traced It was found that 3 4 per cent of the remaining 999 cases were incorrectly certified and that the

remainder were certified on unequivocal evidence (50 per cent), probable evidence (45 per cent), and doubtful evidence (5 per cent)

2 The clinical notes of 879 similar cases recorded in the admission index of the two main hospitals in Leeds from 1950–54 were compared with their death certificates 92 5 per cent were correctly certified at death and in 7 5 per cent the presence of the disease did not appear in the final coding by the Statistics department of the Medical Officer of Health for Leeds

The reasons for the failure of cancer of the lung to appear in the coding might be (*a*) the position of the words " cancer of the lung " on the certificate , (*b*) failure to record carcinoma of the lung on the certificate at all , or (*c*) the fact that the cancer of the lung was an incidental finding not causing death More cases were diagnosed clinically and not certified at death, than were certified at death and not diagnosed clinically

3 Analysis of the cases subjected to autopsy supported the finding that false positive certification was infrequent

4 No sex difference in accuracy of certification was established

We wish to record our thanks to Professor J S Young, University of Aberdeen, for the original suggestion that this investigation should be made, and to Dr R Doll for help and advice We are also indebted to the Consultants in charge of patients in the hospitals in Leeds and elsewhere, to the hospitals Records Officers (in particular to Mr Teale of the Leeds General Infirmary), and to secretaries of departments for help in searching for records We owe a special debt to the Chief Statistical Clerk of the Statistics Department of the Medical Officer of Health, Leeds, to the Chairman and Secretary of the Leeds Executive Council and to many general practitioners for their constant help and willing co-operation Acknowledgement is due to the staff of the Medical Statistics Department of the Registrar-General, Somerset House, for their help in the special search

REFERENCES

Bonser, G M and Thomas, G M —(1955) *Schweiz Z Path* , **18**, 885

McKenzie, A —(1956) *Brit med J* , ii, 204

Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death 6th Revision, Adopted 1948 Vol I

Symposium on the endemiology of cancer of the lung —(1953) *Acta Un int Cancr* , **9**, 440

Willis, R A —(1948) 'Pathology of Tumours ' London (Butterworth and Co Ltd) p 69

Addendum

Since the above communication went to press, an interesting comparison of diagnosis before and after post mortem has been made by the Registrar-General, who arranged for death certificates to be completed by a clinician immediately after death and by a pathologist immediately after post mortem in 1,404 cases in 10 hospitals in England These represented 81 2 per cent of all deaths in these hospitals for the period under review, There was agreement in 51 per cent of cases, while in another 28 per cent the disagreement was one of opinion rather than of fact Cancer of the lung was notably under-diagnosed by clinicians, the amount of error being greatest over the age of 65 years

Reference

Registrar General's Statistical Review of England and Wales, 1956 Part III Commentary, p 182

CANCER OF THE MOUTH 11-YEAR FOLLOW-UP OF 800 CASES

D E STURDY

From The Royal Marsden Hospital, London

Received for publication January 21, 1959

In 1950 Ledlie and Harmer published a report on 800 patients with cancer of the mouth seen at the Royal Marsden Hospital (then the Royal Cancer Hospital) between January, 1936, and December, 1945, with a minimum follow-up period of 3 years These same patients have been reviewed again up to the end of December, 1956, the minimum follow-up period being 11 years The success or failure of the first treatment is reassessed in terms of recurrences

GENERAL REVIEW OF 800 CASES

In the 10 year period 1936–1945 inclusive 800 new cases were seen and these were grouped into 9 sites The number of cases included in each site is shown in Table I and the number of survivors at 5 and 11 years is indicated in the second and third columns of this Table

TABLE I —*The Cases by Sites*

Primary sites	Number of new cases, 1936–45	Number of survivals, minimum 5 years	Number of survivals, minimum 11 years
1 Mucosal surface of lips	160	59	28
2 Mucosa of upper alveolus and hard palate	40	10	5
3 Mucosa of lower alveolus	54	8	4
4 Buccal mucosa	59	11	1
5 Floor of mouth and inferior surface of tongue	136	18	7
6 Dorsum and borders of anterior two thirds tongue	150	25	10
7 Posterior third tongue (and whole tongue)	75	6	2
8 Anterior faucial pillar and soft palate	85	6	4
9 Tonsil proper	41	6	4
	800	149=18 8%	65=8 1%

The age groups, duration of symptoms before treatment, staging, histology and multiplicity of primary tumours were discussed in the previous paper (Ledlie and Harmer, 1950) and the results of the various treatments employed were evaluated In this present paper it is intended to analyse further those patients who were still surviving in December 1950, giving a minimum 5-year follow-up of all cases in the previous paper, and to correlate the survival time with the staging, histology, first treatment and subsequent treatments of each case individually In order to simplify the tables in the previous paper (1950) the primary

sites were condensed into 4 groups and the number of cases in each group at that time is indicated in Table II The right hand column of Table II shows the number of cases considered in this paper, namely 149

TABLE II —*Primary Sites in 4 Groups*

	Number of cases in original paper		Number of cases in present paper (5 year survivals)	
Lip		160		59 (Table III)
Anterior two thirds of tongue		150		25 (Table IV)
Other buccal sites				
Upper alveolus and hard palate	40		10	
Lower alveolus	54	289	8	47 (Table V)
Buccal mucosa	59		11	
Inferior surface of tongue and floor of mouth	136		18	
Oro pharyngeal sites				
Posterior one third tongue	75		6	
Anterior faucial pillar and soft palate	85	201	6	18 (Table VI)
Tonsil	41		6	
		800 cases		149 cases

Multiple Primaries

The incidence of second primary tumours in Ledlie and Harmer's paper was 3 75 per cent, i e , 30 patients in 800 cases The majority of the new primary tumours occurred in the digestive tract and most often between the lip and cardiac sphincter of the stomach In the present paper 12 patients out of the 149 who have survived since 1950 developed a new primary tumour, an incidence of 8 per cent This suggests that patients with an oropharyngeal carcinoma are particularly liable to develop a further primary tumour if they survive long enough

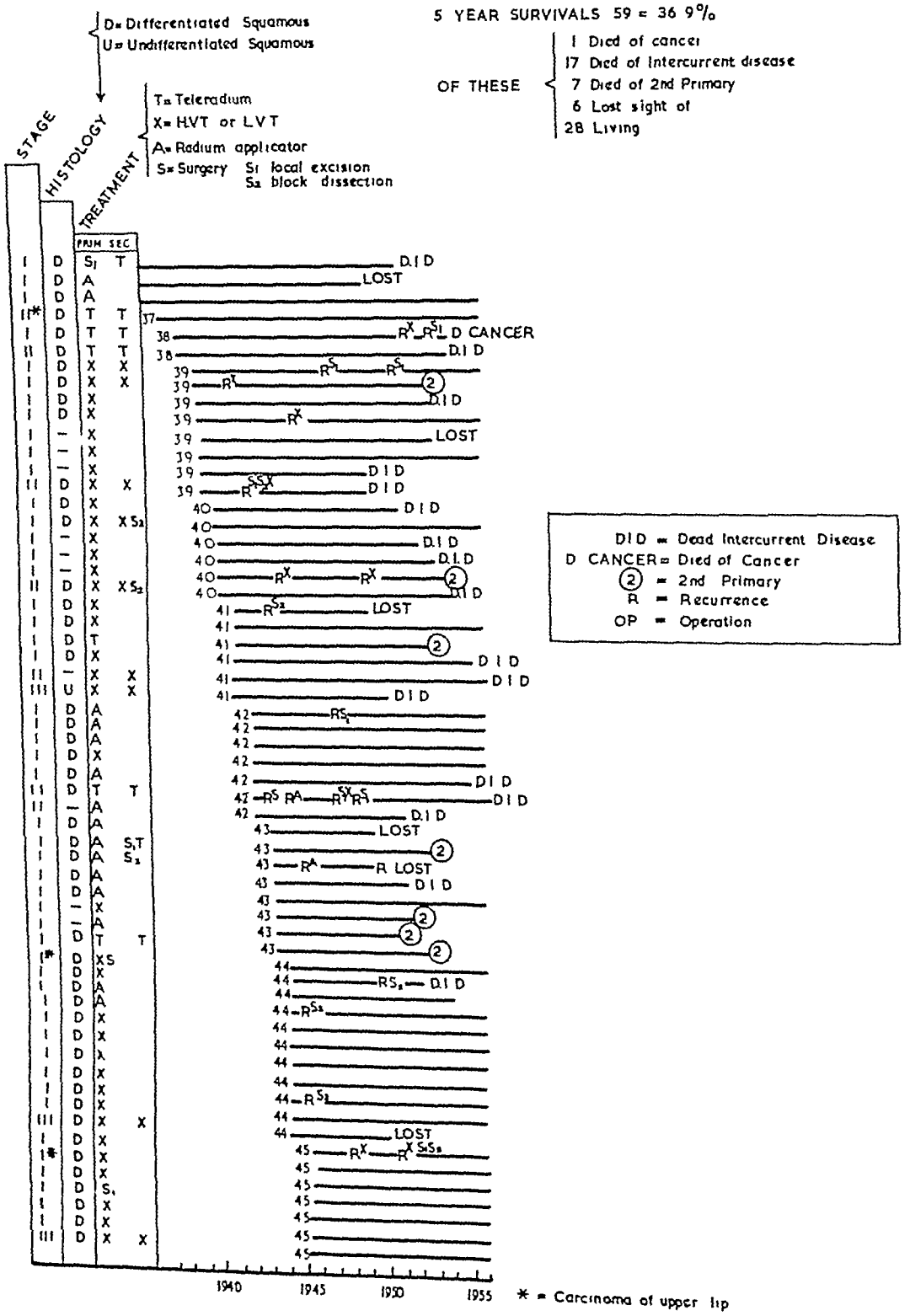
Mucosa of Lip (Table III)

The interesting feature of this group is that only one patient of the 59 considered died of his disease (a Stage I case who recurred after 13 years and who died 2 years later) Fourteen patients, however, had a recurrence of their tumour treated at one time or another The good results for carcinoma of the lip may be attributed not only to its natural history but also to its accessibility for treatment by radiotherapy or surgery and to regular follow-up attendance There was a high incidence of death from a new primary tumour elsewhere in the body, 7 cases occurring in this group—3 in the colon, 2 in the stomach and one each in the breast and bronchus

In Stage I local excision of the primary growth was only employed on three occasions and these patients are recurrence-free after 14, 11 and 10 years respectively Fifteen patients were treated by radium applicator and three of these recurred—two at the primary site and one in the cervical lymph nodes These recurrences were treated by excision, radium needle implantation and block dissection of the neck respectively These patients are alive and well 14, 12 and 6 years after first treatment

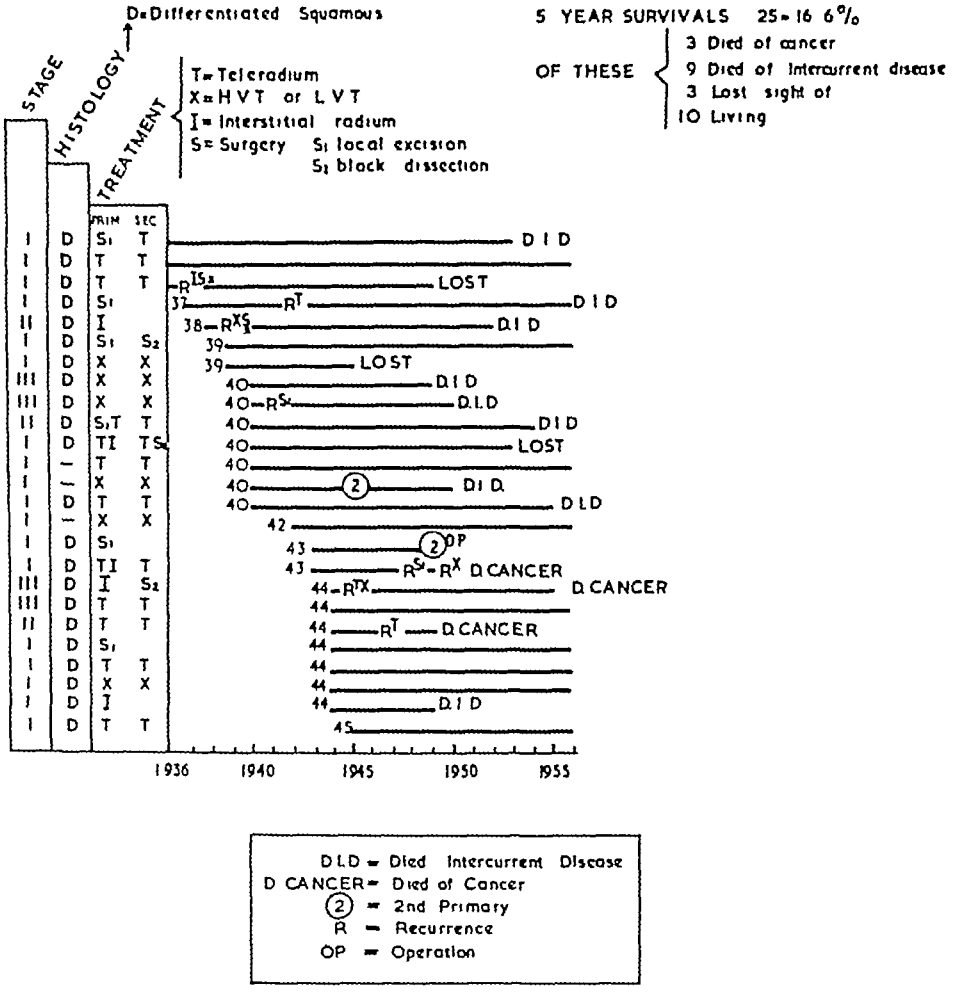
Forty-one patients were treated by external irradiation (superficial X-ray therapy 35 teleradium 6) and the results have been good, only one patient dying of recurrence after 13 years Seven Stage II and 3 Stage III tumours

TABLE III —*Mucosa of lip* 1936–1945 160 *Cases*



were treated by external therapy, one Stage II case also having a block dissection None of these patients has died of cancer External irradiation is very effective in the treatment of carcinoma of the lip in all stages, though wide local excision may be equally effective in Stage I cases Radium therapy has given disappointing results in terms of local recurrence.

TABLE IV —*Anterior two thirds Tongue* 1936–1945 150 *Cases*



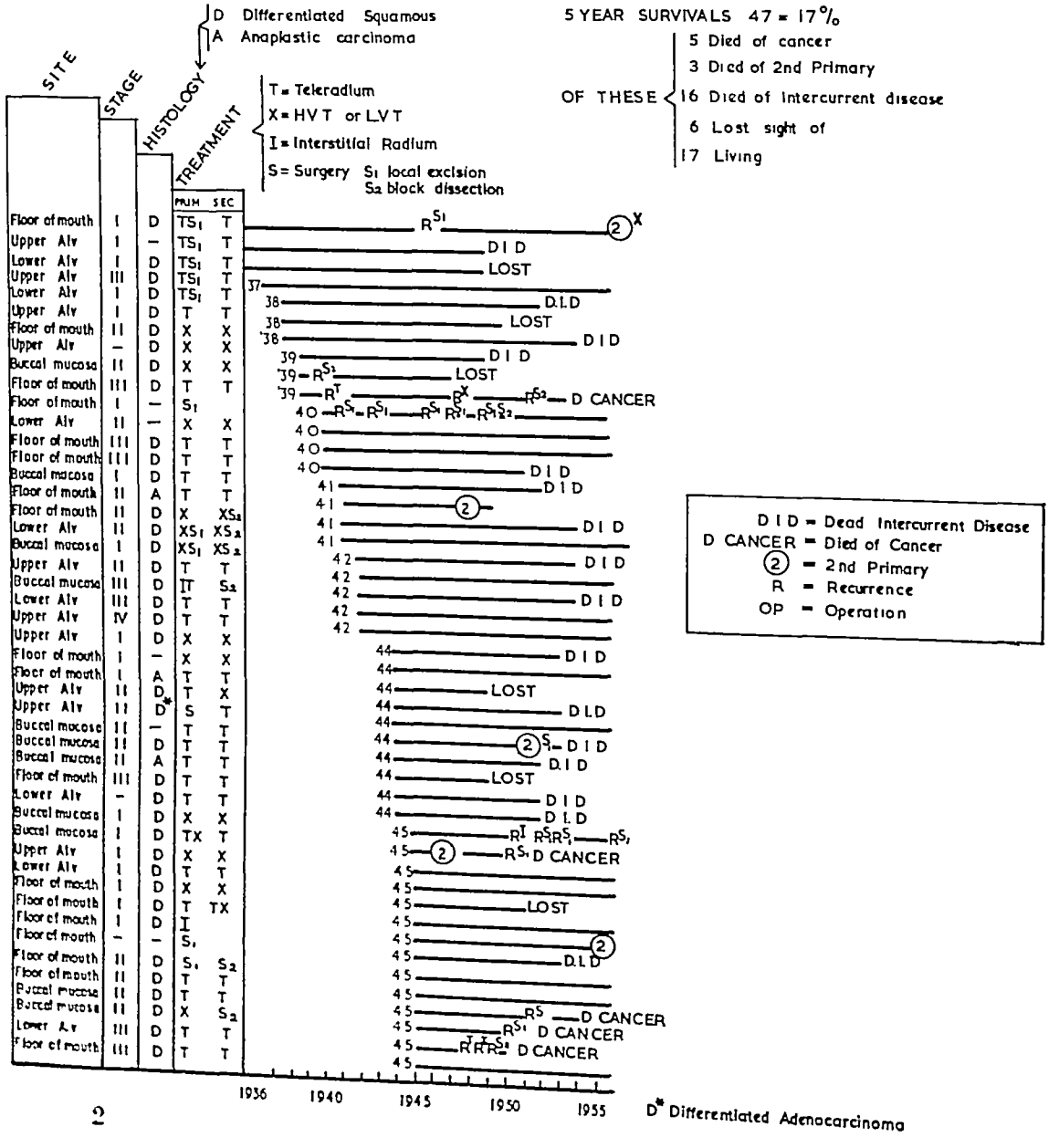
Anterior Two-thirds tongue (Table IV)

Interstitial radium is employed frequently in the treatment of cancer at this site None of the patients still living, however, were treated by this method and 2 of the 3 cases who died of cancer received interstitial therapy in the first instance, combined in one case with teleradium and in another with a block dissection of the neck Seven of the 9 patients still living were treated by external irradiation, the other 2 (Stage I cases) having a local excision, combined with a block dissection of the neck in one case A wide local excision can cure the primary disease, teleradium and X-ray therapy gave good results and proved more effective than interstitial irradiation in this series, although the results of the latter treatment in some other centres do not confirm this (*vide* Table XV of Ledlie and Harmer's paper, 1950)

Upper Alveolus, Hard Palate, Lower Alveolus, Buccal Mucosa, Floor of Mouth and inferior Surface of Tongue (Table V)

Five patients in this group died of their disease and all were treated with external irradiation (teleradium 4 X-ray therapy 1), 2 also having a block dissection of the neck Only one of these, however, was Stage I when first seen It is interesting to note that 3 patients in this group died of a new primary tumour in an area remote from the treated cancer, namely, bronchus, stomach and colon Two of the cases demonstrate well the importance of regular follow-up for all cancer patients and these patients owe their survival to the combined vigilance

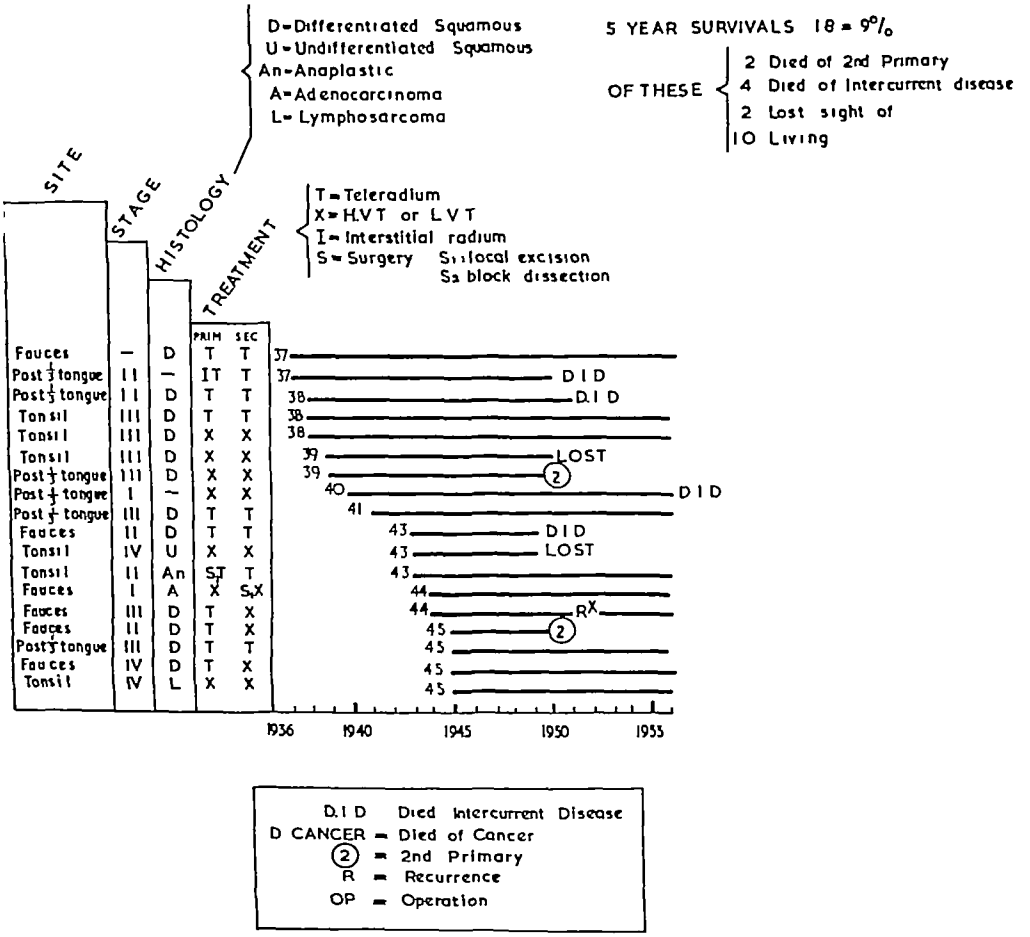
TABLE V —*Upper Alveolus and Hard Palate, Lower Alveolus, Buccal Mucosa, Floor of Mouth and Inferior Surface of Tongue* 1936–1945 289 *Cases*



of the surgeon and radiotherapist One case in particular had five local excisions and finally a block dissection and has now been recurrence-free for 7 years

Seventeen patients in this group are still living and of these 12 had external irradiation (teleradium 8 X-ray therapy 4), 3 were treated by combined methods i e , local excision and X-ray therapy, and 2 had a local excision and block dissection of neck Interstitial therapy was only employed twice for the primary lesion, both of these patients surviving more than 5 years without recurrence Combined methods of treatment have been preferred in Stage II and III cases as opposed to surgery alone One half of the patients still living after external irradiation only were in Stages II, III or IV Some of these patients would be regarded as surgically incurable and the results of radiotherapy are encouraging To summarize most of the cases in Stage II and III who are still living were originally treated by external irradiation (teleradium in almost all cases) It should be stressed, however, that 4 out of 5 cancer deaths were in Stages II and III and were treated primarily by radiotherapy, combined with a block dissection of the neck on one occasion

TABLE VI —*Posterior one third Tongue, Anterior Faucial Pillar, Soft Palate and Tonsil* 1936–1945 201 *Cases*



Posterior One-Third Tongue, Anterior Faucial Pillar, Soft Palate and Tonsil Proper (Table VI)

There are two striking features in this group, firstly the high incidence of survivors in Stages II, III and IV, only 2 being in Stage I, with one patient unstaged, and secondly that only one patient recurred later than 5 years after first treatment All the 18 cases considered were treated with external irradiation (teleradium 11 X-ray therapy 7), one case also having a local excision and another interstitial irradiation None of this group eventually died of the disease, 2 died of a new primary growth elsewhere Histologically 12 out of the 18 cases had well-differentiated squamous cell carcinoma Radiotherapy is the treatment of choice for all cases in this group, but nevertheless, only 9 per cent of patients treated survived 5 years

CONCLUSIONS

The conclusions made by Ledlie and Harmer in their 1950 paper were —

(1) Although very different methods of treatment may be employed the results are much the same

(2) Carcinoma of the lip is best treated by superficial X-ray therapy

(3) Small Stage I growths in accessible situations within the mouth may be widely excised, but can be treated with equal success by radiotherapy Regular follow-up is particularly essential in these cases

(4) Recurrence at the primary site is more common than is usually appreciated and may be as frequent as recurrence in the nodes after both have been apparently successfully treated

(5) In most buccal and oro-pharyngeal sites the results of teleradium therapy are superior to those of X-ray therapy

(6) The results of teleradium treatment for growths of the anterior two-thirds of the tongue has not been so successful as interstitial methods reported from other centres

These conclusions were formulated on a minimum 3-year follow-up and remain largely true in the light of this more recent survey with a minimum 11-year follow-up period Point (6) in the conclusions, however, has not been re-examined Between 9 and 12 per cent of patients were lost to follow-up in the whole series This figure would probably be improved upon at the present time, but many cases failed to attend during the war years, 1939–1945, and were consequently lost for follow-up purposes

The conclusion of the original paper, that for treatment to be successful it must be radical in the first instance is supported and in addition it is confirmed that the only method of ascertaining complete eradication of the disease is regular out-patient follow-up

I wish to acknowledge my debt to the staff of the Royal Marsden Hospital for permission to publish their clinical material I am grateful for help from Miss June Akister who constructed the Tables and Miss Susan Taylor of the Records Department

REFERENCE

LEDLIE, E M AND HARMER, M H —(1950) *Brit J Cancer*, 4, 6

A COMPARISON OF TREATED AND UNTREATED CASES OF CANCER OF THE BREAST

A J PHILLIPS

From The National Cancer Institute of Canada, 800 Bay Street, Toronto 5, Ontario, Canada

Received for publication January 31, 1959

Whether or not treatment of cancer of the breast materially alters the survival rate is a problem which has given rise in recent years to much speculation and several papers in the medical literature The evidence for treatment has been indirect, being based upon comparisons with groups of untreated patients not directly comparable with the treated groups From a statistical point of view the best method of attacking the problem would be to treat alternate patients but for numerous reasons this is impossible Another method, statistically acceptable, is to match each untreated patient with a treated one Such a matching technique has been employed in the present paper

This study includes 230 cases of untreated cancer of the breast which were collected from cancer treatment centres in Canada, England, France and the United States For each case the following data were requested —

(*a*) age at diagnosis
(*b*) duration of symptoms before diagnosis
(*c*) pathological diagnosis
(*d*) clinical findings
  (1) confined to breast (operable)
  (2) confined to breast and axilla (operable)
  (3) confined to breast or breast and axilla but inoperable because of fixation to chest wall or fixation of axillary lymph nodes
  (4) evidence of secondary deposits other than same breast or axilla
(*e*) reasons for no treatment
  (1) patient refused
  (2) patient too old
  (3) intercurrent disease
  (4) disease too advanced
(*f*) survival, from diagnosis

Table I shows the age distribution of the 230 untreated cases compared with two treated series, one of 783 cases from the Toronto General Hospital in Ontario (Phillips, 1954, unpublished study) and one of 993 cases from the province of Saskatchewan (Watson, 1951) It will be noted that approximately 60 per cent of the untreated cases are over 60 years of age with an average age of 64 years This average age is high compared to the two treated groups, illustrating one of the problems in comparing series of cases

Data on duration of symptoms were given for 181 of the untreated cases The average for these 181 cases was 27 1 months with the longest being 204 months and the shortest one month

TABLE I —*Age Distribution of Untreated and Treated Cases of Cancer of the Breast*

Age group	Proportion in each age group		
	Untreated (%)	Ontario (%)	Saskatchewan (%)
Under 30	0 4	1 4	1 9
30–39	4 4	8 6	10 1
40–49	12 2	22 0	26 1
50–59	20 2	26 2	33 5
60–69	25 4	24 4	19 2
70–79	25 2	14 1	8 1
80 plus	12 2	3 3	1 1
Average age	64 years	56 3 years	52 years

Table II shows the extent of the disease in the untreated group as determined by clinical examination It will be noted that the 84 cases in whom the disease was confined to the breast and to the breast and axilla have been classified as " operable " These cases have received further study later in this report

TABLE II —*Extent of Disease as Determined by Clinical Examination in Cases of Untreated Breast Cancer*

Extent of disease	Number of cases	Per cent
Confined to breast (operable)	43	18 7
Confined to breast and axilla (operable)	41	17 8
Fixation to chest wall or of axillary lymph nodes	32	13 9
Secondary deposits of disease	106	46 1
Not specified	8	3 5
	230	

Pathological proof of malignancy was obtained on 46 cases or 20 per cent of the untreated series Of the remaining 184 cases, a biopsy was not taken in 176 and in 8 information on this point was not given

Table III shows the various reasons why these patients were not treated and it will be noted that the two main reasons were that the disease was too advanced or the patient refused treatment

TABLE III —*Reasons for not Treating Cases of Untreated Breast Cancer*

Reasons	Number of cases	Per cent
Patient refused	84	36 5
Patient too old	19	8 3
Intercurrent disease	5	2 2
Disease too advanced	101	43 9
Other	11	4 8
Not specified	10	4 3
	230	

Two measures of survival have been calculated for this group of untreated patients The first of these is the survival from the date of examination or diagnosis

and the second from the date of alleged onset of symptoms These data are shown in Table IV

TABLE IV —*Survival Calculated from Date of Diagnosis and Date of Onset of Symptoms for Untreated Breast Cancer Cases*

Survival from	Number of cases	Mean survival (months)	Survival (%) 1st yr	2nd yr	3rd yr	4th yr	5th yr	10th yr
Date of diagnosis	230	19 2	37 0	22 5	15 0	11 5	9 5	1 3
Onset of symptoms	181*	46 2	75 2	58 2	44 0	34 0	28 5	6 6

* Data on duration of symptoms available on 181 cases only

The average duration of life from onset of symptoms, 46 2 months, compares with 40 5 months quoted by Daland (1927), 39 8 months quoted by Lazarus-Barlow (1924), 39 6 months quoted by Wyard (1925) and 38 0 months quoted by Wade (1946)

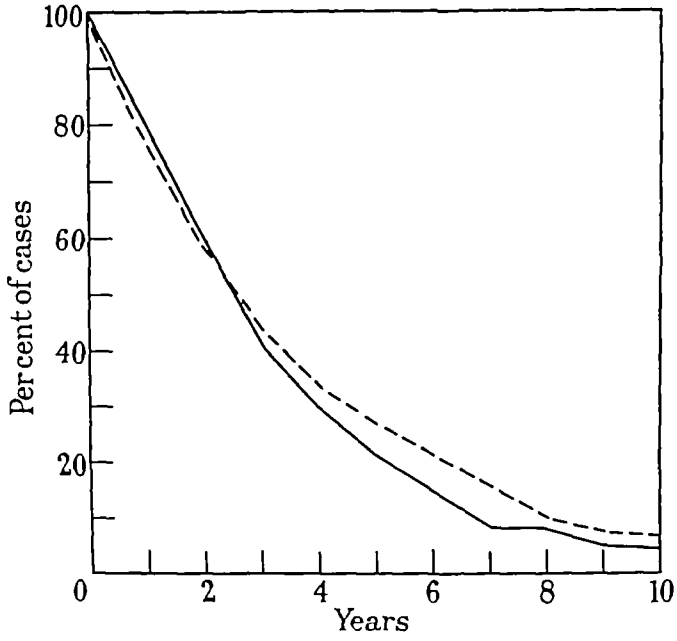


FIG 1 —Comparison of survival rates from onset of symptoms in two series of untreated breast cancer patients ———— Daland (1927) - - - - Present series

In Fig 1 the annual survival rates from onset of symptoms have been compared with those reported by Daland (1927) and it will be noted that the two groups of cases show approximately identical trends

Comparison with Treated Patients

The relative value of treatment in breast cancer has been assessed by matching each untreated operable case with a treated one from the Toronto General Hospital Patients were matched on age at diagnosis and on age at alleged onset of symptoms and a variation of one year was permitted Since many treated patients were of the same age as the untreated one, the selection of the treated patient was made by using the table of random numbers The extent of disease in the matching

process was controlled since operable cases in the untreated series were matched with Stages I and II in the treated group where —

STAGE I is described as the tumour localized in the breast, skin not involved and no metastases evident in the axillary lymph nodes or elsewhere

STAGE II is described as the tumour localized in the breast, moveable, skin not involved and axillary lymph nodes involved but no other evidence of metastases

In the untreated series 84 cases were classified as operable and these have been matched with 84 treated cases on the basis of age at diagnosis The duration of symptoms was not given on 19 of the untreated operable cases hence the matching on the basis of age at alleged onset of symptoms was made on 65 cases For those patients matched on age at diagnosis the survival rates have been calculated from date of diagnosis and are shown in Table V and Fig 2 The differences

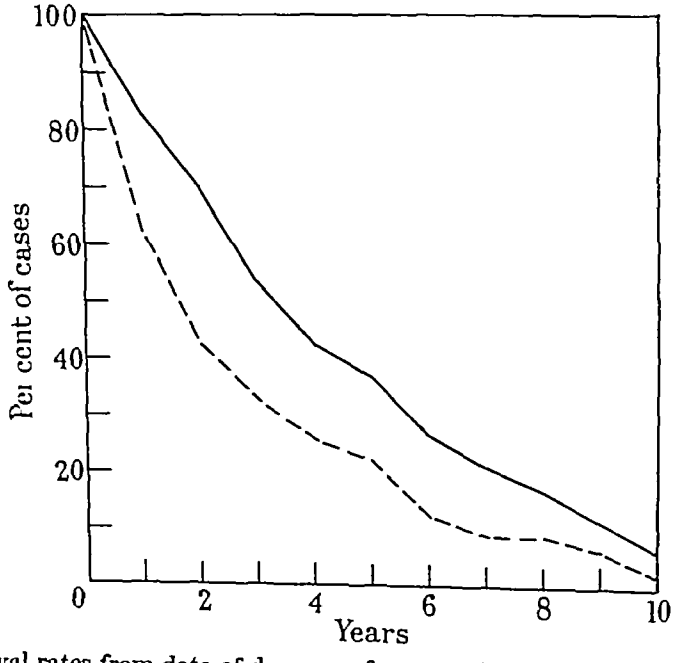


FIG 2 —Survival rates from date of diagnosis for treated and untreated patients matched for age at diagnosis and extent of disease ———— Treated – – – – Untreated

TABLE V —*Comparison of Survival Rates from Date of Diagnosis for Treated and Untreated Patients Matched for Age at Diagnosis and Extent of Disease*

Time (years)	Untreated (N = 84) Number surviving	Per cent	Treated (N = 84) Number surviving	Per cent	Difference Significant*
1	53	63 1	70	83 3	"
2	36	42 8	59	70 2	"
3	28	33 3	45	53 6	"
4	22	26 2	36	42 8	"
5	19	22 6	31	36 9	"
6	11	13 1	23	27 4	"
7	8	9 5	18	21 4	"
8	8	9 5	14	16 7	"
9	6	7 1	10	11 9	Not significant
10	2	2 4	6	7 1	" "

* At the 0 05 level of confidence

between the proportions of patients surviving at each year have been tested for significance by the chi-square technique It will be noted that the proportion of treated patients surviving is significantly greater than untreated at each year to eight years

The survival rates for untreated and treated cases matched for age at onset of symptoms have been calculated from the date of onset of symptoms and are shown in Table VI The significance of the differences in the proportion surviving at each year have been tested by the chi-quare method It will be noted that the differences are not significant except at the two-year point

TABLE VI —*Comparison of Survival Rates from Onset of Symptoms for Treated and Untreated Patients Matched for Age at Onset of Symptoms and Extent of Disease*

	Untreated ($N = 65$)		Treated ($N = 65$)		
Time (years)	Number surviving	Per cent	Number surviving	Per cent	Difference
1	54	83 1	60	92 3	Not significant*
2	45	69 2	57	87 7	Significant
3	43	66 1	48	73 8	Not significant
4	33	50 8	39	60 0	,, ,,
5	29	44 6	30	46 1	,, ,,
6	22	33 8	23	35 4	,, ,,
7	14	21 5	20	30 8	,, ,,
8	9	13 8	18	27 7	,, ,,
9	8	12 3	14	21 5	,, ,,
10	6	9 2	12	18 5	,, ,,

* At the 0 05 level of confidence

DISCUSSION

The purpose of studying untreated cases of breast cancer is two-fold since it provides information on the natural history of the disease as well as an opportunity to assess the efficacy of treatment However, the number of such cases is limited since patients who do not receive surgery or radiation therapy often receive hormone therapy and therefore must be considered as having been treated Further, those who are untreated are often not followed hence the survival time is unknown With these limiting factors it is felt that the present series of 230 cases constitutes a substantial sample of this type of cancer patient

Since the average age of this untreated group is considerably greater than that of treated groups one is not justified in comparing the untreated series with a treated one To overcome this problem and to ensure that some measure of similarity exists between the two groups it becomes necessary to consider matching patients In the matching process three variables have been considered, age at diagnosis, extent of disease and duration of symptoms as determined by age at alleged onset of symptoms The validity of data on alleged duration of symptoms has been questioned repeatedly In the untreated cases being studied the average duration of symptoms was 27 1 months which is considerably greater than that reported in treated series and the lack of significance in the survival from onset of symptoms between treated and untreated patients may be due to inconsistencies in these data

Another factor which affects the matching of untreated and treated patients is the proportion of cases in each group having pathological proof of malignancy

In most treated series the proportion is approximately 75 per cent and it is probably closer to 100 per cent in the matched treated patients being considered in this paper On the other hand only 17 5 per cent of the matched untreated cases were diagnosed pathologically This weighting of untreated cases on the side of clinical diagnosis as opposed to microscopic could favour the untreated group by including lesions which would be diagnosed microscopically as benign

SUMMARY

A study has been made of 230 cases of untreated breast cancer

The natural history of the disease as revealed by these cases has been compared with an earlier series by Daland (1927)

The average age and average duration of symptoms for the untreated cases were greater than those reported for treated cases

Untreated operable patients have been matched with treated operable patients from the Toronto General Hospital, Ontario, on the basis of age at diagnosis and age at onset of symptoms

In comparing treated and untreated patients, matched for age at diagnosis it was found that —

(*a*) the survival rate calculated from date of diagnosis was significantly greater for treated patients than for untreated ones for each year to eight years

In comparing treated and untreated patients, matched for age at onset of symptoms, it was found that —

(*b*) the survival rate calculated from onset of symptoms was consistently higher for treated patients than for untreated ones but the differences were not significant

REFERENCES

DALAND, E M —(1927) *Surg , Gynec Obstet* 44, 264
LAZARUS-BARLOW, W S —(1924) *Brit med J* , II, 266
WADE, P —(1946) *Brit J Radiol* , 19, 272
WATSON, T A —(1951) ' Results of Treatment of Cancer in Saskatchewan ', Regina (Saskatchewan Cancer Commission)
WARD, S —(1925) *Brit med J* , I, 206

EARLY DIAGNOSIS OF MALIGNANT CONDITIONS IN LYMPH NODES

L KREYBERG AND O H IVERSEN

From the Institutt for Generell og Eksperimentell Patologi, Universitetet i Oslo

Received for publication November 1, 1958

The diagnosis of a malignant condition is always of very serious significance. If the diagnosis is correct, the future life of the patient is troubled and the ultimate fate is dark

But, even if the diagnosis is incorrect, it is accompanied by grave consequences The patient is stigmatized, he lives in a tense condition full of anxiety, and he is likely to be subject to radical therapeutic steps, to surgery or intensive irradiation, not seldom followed by a more or less marked reduction of his activity and well-being

In our days the situation is aggravated by the intensive propaganda for " early diagnosis ", as well as by the therapeutic perfectionism aiming at " cure " The plea for early diagnosis by its own momentum leads to a diagnosis supported by small deviations from the normal, small in quality and small in quantity The " earlier " the changes, the greater the uncertainty

This problem often is presented — which are the therapeutic steps to be taken on the basis of uncertain clinical and histological data ? In few fields is this uncertainty better felt than when lymphoid tissue is involved

In the present paper an analysis will be made of a series of cases, where the clinician, in the presence of enlarged lymph nodes, decided to have a biopsy examined—and where, after the examination, the pathologist gave a diagnosis of — " Lymphoid hyperplasia Malignancy cannot be excluded "

The material is part of a total of 3483 lymph nodes received at our laboratory in the years 1946–55 Out of this series 124 cases were found, presenting the criteria mentioned

The primary diagnosis in the following is the designation for the original diagnosis, with the accompanying remark These diagnoses were, during a period of 10 years, given by 5 different pathologists The diagnostic criteria, the descriptions and the terminology are accordingly not uniform

The 124 cases can, corresponding to the terms and expressions used, be divided into two grades of uncertainty Grade II means that the suspicion of malignancy was moderate Grade III means that the suspicion was grave

This material was now made the subject of a *revised diagnosis*, performed by one pathologist (K), according to uniform criteria and without any knowledge of the clinical information or of the previous (primary) diagnosis

Also this material was now " graded ", with Grade I meaning that the morphological criteria does not substantiate a diagnosis of malignancy Grade IV means an unreserved diagnosis of malignancy Also this material will have Grades II and III, indicating a slighter or a graver suspicion of malignancy These Grades II and III are not based upon the same criteria as those of the primary diagnosis, but, it is nevertheless regarded as useful to correlate these grades

The patients were clinically re-examined and their fates registered In every

case satisfactory information was obtained Very helpful, as usual, was our Cancer Registry We are likewise indebted to the clinicians who generously gave us their help

More than $\frac{2}{3}$ of the cases (86) had an observation period of 5 years, or more, and all cases had an observation period of 3 years or more

As our study revealed that the patients dying from, or with, a malignant lymphoid disease had an average survival time of 2 57 years and a median survival time of 1 25 years after the biopsy, we regard a limit of 3 years as acceptable

TABLE I —*The Result of the Clinical Follow-up*

Clinical development	Number		
	M	F	Total
Symptom free	29	20	49
Died from intercurrent disease	2	3	5
Suffering from malignant lymphoid condition	10	5	15
Died from or with malignant lymphoid condition	39	16	55

TABLE II —*The Final Clinical Diagnosis in Cases of an Accepted Malignant Condition*

	Died			Alive		
	M	F	Total	M	F	Total
Lymphoid leukaemia	18	6	24	2	1	3
Lymphosarcoma	16	7	23	5	3	8
Giant follicular hyperplasia	1	0	1	0	0	0
Reticulosarcoma	1	2	3	1	0	1
Hodgkin's disease	3	1	4	2	1	3

Correlation of primary histological diagnosis and final clinical development

As already mentioned, during the primary diagnostic work, no grading was performed A grading was, however, done during the present study and based upon the original phrasing of the suspicion of malignancy

Grade II, representing cases with a moderately worded suspicion of malignancy, embraces 41 patients Two patients died from an intercurrent disease 1 and 11 years after the biopsy was made Of the remaining 39 patients 7 (18 per cent) developed a malignant lymphoid condition, the others living symptom free

It may be mentioned that every one of these 7 malignant cases was correctly diagnosed during the revision This indicates that the histological material actually was representative The suspicion of malignancy was substantiated in 18 per cent of the cases only

When 34 patients were under a certain suspicion of malignancy after examination of the morphological material, in spite of the later benign clinical course, this indicates that the diagnosis had been built upon very small deviations from the normal, and that the pathologists involved probably had been influenced either by the case history or by a philosophy of aggravating the diagnosis for "safety sake"

Grade III, representing cases with a more strongly worded suspicion of malignancy, embraces 83 patients Three patients died from intercurrent disease $\frac{1}{2}$, $\frac{1}{2}$ and 1 year after the biopsy Out of the remaining 80 cases a malignant lymphoid condition was diagnosed in 63, that is in $\frac{4}{5}$ of the cases Among the 17 symptom free, there are 5 patients with only 3 years of observation, so that a few more may develop malignancy Two only, of the 17 living symptom free, were

given specific treatment A postulated " cure " can therefore not materially alter the evaluation

Correlation of the revised histological diagnosis and the final clinical development

Grade I represents cases where, during revision, the morphological findings did not substantiate a diagnosis of malignancy, a total of 40 cases Four patients died from intercurrent disease ½, 1, 1, and 11 years after the biopsy Among the remaining 36 cases 2 only showed development of a malignant lymphoid condition (5 per cent) Both these cases were from the primary diagnosis classified as Grade III

Case 1 A 14 years old boy, with marked clinical symptoms mentioned in the case history submitted to the pathologist The slide has been restudied several times, but we cannot find a sufficient morphological basis for a diagnosis of malignancy Most probably the diagnosis was made on non-representative material and a suggestive case history The lymph nodes had been irradiated

Case 2 A 61 years old man dying of lymphatic leukaemia within one year after the biopsy The slide has been re-examined several times and is regarded as difficult to interpret owing to a considerable admixture of plasma cells and eosinophiles In this case a diagnostic failure must be admitted

Grade II embraces only 4 patients, 2 living symptom free, 2 developing a malignant lymphoid condition

In both cases of malignancy, the lymph nodes were small, the changes minor, but in both cases lymphocytic infiltration of the capsule was present These two cases should have been referred to Grade III

Grade III embraces 24 patients out of which 19 (⅘) showed a malignant lymphoid condition, whereas 5 patients remained symptom free in the period of observation 10, 8, 7, 5 and 3 years after the biopsy

In the slides from these patients four cases gave the picture of a well conserved architecture of the lymph nodes, but the follicles were considerably enlarged and showed a great number of mitoses This finding will be commented upon later

In the fifth case the histological picture was very like that of the case in Grade II where leukaemia developed later Here again the criteria did not permit a correct diagnosis

Grade IV, representing cases where the malignancy diagnosis was now given unreservedly, embraces 56 cases, with one patient dying from an intercurrent disease ½ year after the biopsy Of the 55 remaining, 47 showed a malignant lymphoid condition, whereas 8 remained symptom free One of the latter suffered from infectious mononucleosis, which means a definite complicating factor for a correct diagnosis In the other cases the histological picture was still after several revisions regarded as being of a grave character The observation periods were one case 11 years, two cases 8 years, one case 7 years, one case 4 years, and two cases 3 years

TABLE III —*Results of Histological Grading*

Malignancy grade	Series: Primary		Series: Revised	
I	—		40	
II	41	124	4	28
III	83		24	
IV	—		56	

The first striking result of the revision is that from the 124 doubtful diagnoses 28 only (22 5 per cent) are left as such The others have been placed with diagnoses with or without the affix malignancy, without a question mark This means that the revised diagnosis through its pertinent criteria is more categorical The differentiation is greater But, nothing is thereby implied as to the correctness of the diagnosis The relative merits of the two diagnostic series will have to be further analysed

TABLE IV —*Correlation of the Primary and the Revised Histological Diagnosis and Clinical Development*

	Malignancy grade	Symptom free (a)	With malignant lymph manifest (b)	Ratio a	b		
Primary diagnosis	II	32	7	5	1		
	III	17	63	1	4		
Revised diagnosis	I	34	2	17	1		
	II	2	2	1	1		
		7	21			1	3
	III	5	19	1	4		
	IV	8	47	1	6		

The five patients dying from intercurrent diseases have not been included

The most important findings are

(i) The revision of the doubtful cases from the primary diagnostic series has led to a reduction of doubt to one out of five cases only

(ii) The new categorical formulation of the diagnosis in the previous doubtful cases has resulted in a fair accuracy

(1) if the diagnosis is given as benign, there is a 17 to 1 chance that this is correct,

(2) if the diagnosis is given as malignant, there is a 6 1 chance that this is correct,

(3) where doubt is still entertained, there is a considerable chance (1 1 Grade II, 4 1 Grade III, average 1 3) that the condition under diagnosis is malignant

These figures, taken with the limitation of the number of cases, give a measure of the accuracy of our diagnosis in such cases

Before we discuss the implications of these facts, a short survey is given of the criteria found to be of greatest value in forming our diagnosis of the material under discussion

Evaluation of the morphological criteria

In Table V the histological features are arranged in relation to the clinical development The features are listed in such a manner, that those showing the greatest correlation with benign lymphoid conditions are on the top, and those connected with malignancy at the bottom of the table In order to ease the comparison the occurrence is given in per cent

Based upon these figures, a relative occurrence between the benign and the malignant conditions of each of the features examined has been calculated If, for instance, ' dominance of small lymphocytes " occur among the symptom-free

TABLE V —*Histological Criteria and Diagnostic Significance in Regard to Benign and Malignant Lymphoid Conditions*

Criterion	Non malignant cases	Value in favour of benignancy	Patients with malignant lymphoid conditions	Value in favour of malignancy
No capsular, or pericapsular infiltration	**56**	**95**	3	5
Architecture conserved	**74**	**74**	26	26
Dominance of small lymphocytes	**76**	**72**	29	28
Reticulum cell proliferation	36	65	19	35
Polymorph cell population	24	60	16	40
Fibrosis	26	58	19	42
Large follicles	24	55	20	45
Dominance of medium size lymphocytes	20	36	36	64
Increased number of mitoses	10	32	21	68
Capsular, or pericapsular infiltration	42	30	97	70
Obliteration of sinus	22	23	**74**	**77**
Architecture destroyed	10	19	**44**	**81**
Dominance of lymphoblasts	2	5	**36**	**95**

patients 76 times and among the patients suffering from a malignant lymphoid condition 29 times, this means that the relative occurrence in benign cases is $\frac{76 \times 100}{105}$, that is 72 per cent

As would be expected, the commonest features in the benign conditions are those regarded as belonging to the normal lymph node As likewise would be expected from the very selection of cases, no case presented only normal features

The most important features pointing in the direction of a benign condition in the lymph node are lack of infiltration of the capsule and the pericapsular tissue, conservation of the general architecture and dominance of small lymphocytes

The findings indicate that if two, or especially three of these features are present, there is a very high probability that the patient is not suffering from a malignant lymphoid condition—of course with the proviso that the biopsy is representative A striking illustration of the relevancy of this reservation is shown in Fig 1, where a group of small lymph nodes was received for examination and one only out of the 12 showed malignant changes All the others were normal

The most important feature pointing in the direction of a malignant condition in the lymph nodes are dominance of lymphoblasts, non-conservation of the general architecture and obliteration of the peripheral sinus

The presence of two or all three of these features gives a very high probability of malignancy

As regards the other features, none of them reaches sufficiently high in importance to be of very great diagnostic value

As previously mentioned, one combination ought to be stressed, as already done by Rappaport, Winther and Hicks (1956) namely, large follicles and a great number of mitoses Our findings completely agree that this combination is more linked with a benign condition than with giant follicular hyperplasia We were not sufficiently aware of this situation, hence our classification of these as cases of malignancy (Grade III)

FIG 1.—Twelve small lymph nodes were received, eleven showing the criteria of normal nodes with well conserved architecture and numerous follicles One node only showed obliteration of the normal architecture and a diffuse proliferation of lymphoblasts

GENERAL DISCUSSION

One hundred and twenty four cases of lymphoid hyperplasia, where clinically and morphologically the picture was more or less uncertain, were re-examined clinically and the slides revised

The histological revision resulted in a greater differentiation, inasmuch as $\frac{4}{5}$ of the morphologically uncertain cases were classed as either benign or malignant In $\frac{1}{10}$ of these cases the clinical development actually confirmed the correctness of the revised diagnoses

One fifth of the biopsies remained morphologically uncertain

These figures certainly do not indicate the total precision of the diagnostic service of our laboratory regarding lymph gland material, even as regards the question a benign or malignant condition—as only the initially doubtful cases have been included in the present study

The general findings therefore show that a biopsy in clinically doubtful cases of enlarged lymph nodes is a very important and useful diagnostic means in deciding the presence of malignancy or not

Nevertheless, certain limitations exist First, the biopsy may not be representative This is a general limitation of technical nature

Second, the criteria used are not of decisive validity Pictures resembling malignant conditions may accompany benign diseases Infectious mononucleosis may be mentioned as an example Cases of malignancy may on the other hand be presented for diagnosis in stages so early that the criteria are not sufficiently developed to permit a diagnosis

Third, the present knowledge may not be sufficiently distributed and appreciated, as shown in the cases of large follicles and high number of mitoses

If our results are now placed in relation to the situation very often presented to the clinician and the pathologist, namely, a doubtful case of lymphoid malignancy, we are forced to take a standpoint of grave consequences for the patient on a very uncertain ground

If the clinical picture greatly points in the direction of malignancy (in cases of lymphoid leukaemia a blood examination is very important) and likewise the pathologist is liable to place the case as Grade III or IV, it will be justifiable to accept and treat the patient as one suffering from a malignant lymphoid condition

If, on the other hand, the clinical symptoms are more vague and the pathologist cannot go further than a Grade II suspicion, it may be justifiable not to break the news of a malignant diagnosis to the relatives of the patient, and to delay therapeutic action

The main support for this conservative attitude is the fact, that malignant lymphoid conditions are scarcely ever definitely cured A delay in diagnosis probably does not jeopardize the final chances of the patient

If, on the other hand, the patient is not actually suffering from a malignant condition the stigma with the ensuing psychological stress or shock, the reduction of vitality and the exposure to serious means of treatment are of such grave consequence that they cannot be outbalanced by the possible loss of an " early diagnosis " and thereby an early treatment

This is the philosophy behind our teaching and our discussions with the clinicians in cases of the type discussed in the present paper

In spite of an intensive and often uncritical propaganda, the Norwegian clinicians seem to support this philosophy Of the 41 patients with a primary diagnosis of hyperplasia with a moderate suspicion of malignancy (Grade II) 9 only were given specific treatment, possibly those with the most marked clinical symptoms Five of these 9 are alive symptom-free, 4 have developed a malignant lymphoid condition More important, out of the 32 untreated patients 27 are symptom-free, 2 died from an intercurrent disease and 3 only have manifested a malignant lymphoid condition

It seems that the total benefit of the conservative attitude has been greater than the total possible loss of an early and intensive treatment

For the patient a very close contact between clinician and pathologist is of utmost importance

Dr Iversen has during this study received grants from " Elisabeth og Knut Knutsen O A S Fond for Kreftforskning " and from " Landsforeningen mot Kreft "

REFERENCE

RAPPAPORT, H , WINTHER, W J AND HICKS, E B —(1956) *Cancer*, **9**, 972

TUMOUR CELLS IN THE BLOOD WITH SPECIAL REFERENCE TO PRE- AND POST-HEPATIC BLOOD

W S FLETCHER AND J W STEWART

From the Department of Surgical Studies and the Bland-Sutton Institute of Pathology, The Middlesex Hospital, London, W 1

Received for publication January 29, 1959

BLOOD stream metastasis of malignant tumours has long been recognised Over the years there have been sporadic reports of atypical cells in the blood of cancer patients but it is only with the advent of techniques for isolating different types of cells from whole blood by utilising minor differences in their gravity (Fawcett and Valle, 1952) that it has been possible to demonstrate definitely such tumour cells

Engell (1955) reported that in 59 per cent of patients with carcinoma of the rectum or colon either definite malignant cells, or atypical cells suggestive of malignant cells, were demonstrable in the venous blood draining the tumour area He also found tumour cells in the blood draining other tumour sites and in the peripheral blood Other investigators, using slightly different separation techniques, have confirmed Engell's findings Most noteable of these are Sandberg and Moore (1957) and Sandberg *et al* (1958), whose technique depends on the rapid sedimentation of erythrocytes with bovine fibrinogen, and Roberts *et al* (1958) and Cole *et al* (1958) who refined this technique by layering the tumour cells at an albumin interface of known specific gravity after sedimentation of the erythrocytes with bovine fibrinogen

Our own work falls into two parts We first of all investigated patients with a variety of tumours, but particularly with tumours of the parotid and breast Having found that we were able to identify tumour cells both in the blood draining tumour sites and in the peripheral venous blood, we have attempted to determine to what extent tumour cells are filtered from the blood by the liver in patients either with primary gastro-intestinal carcinoma or with abdominal secondary deposits

METHOD

For the second part of our studies blood was taken before and after it entered the liver Fig 1 demonstrates the way in which the post-hepatic samples were taken A catheter is passed under fluoroscopy through the right side of the heart and into the right and then the left hepatic vein, and a 5 ml sample of blood is taken from each vein At the same time 5 ml sample of peripheral blood is also taken As it is difficult to take pre- and post-hepatic blood simultaneously at operation the post-hepatic is taken pre-operatively The pre-hepatic samples are taken at operation from the portal vein as soon as the abdomen is opened and with minimal handling of the tumour Again a simultaneous 5 ml of peripheral blood sample is taken

The samples are processed immediately using a slight modification of Sandberg and Moore's (1957) technique The blood is put directly into a tube containing 1 mg of heparin and 80 mg of bovine fibrinogen in 2 ml of water, and allowed to stand until the erythrocytes sediment The supernatant fluid is then pipetted off and centrifuged at 1000 r p m for 5 minutes The supernatant plasma is decanted and the residual portion of cells resuspensed in the remaining fluid A known aliquot of this suspension is smeared on four slides and stained with Leishman's stain , two are counter-stained with peroxidase, which provides a

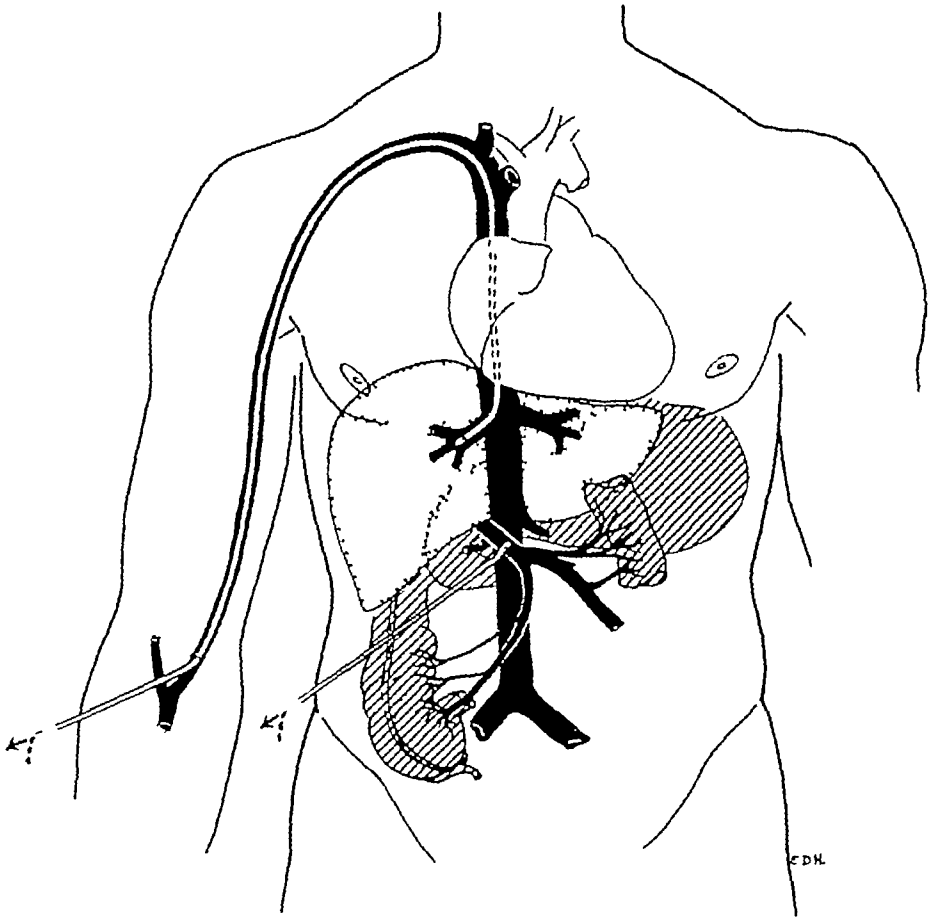


FIG 1 —Diagram showing catheters in position for taking hepatic and portal samples of blood

contrasting background in that neutrophils are peroxidase-positive while tumour cells, lymphocytes and monocytes are peroxidase-negative Examination of approximately one-fourth of one peroxidase, and one fourth of one non-peroxidase stained smear for each sample was then carried out by one of the authors (W S F) and the possible malignant cells marked These were reassessed by the other author (J S) and possible blood cells excluded

The criteria for determining whether cells are tumour cells have been discussed by numerous authors (Engell, 1955 , Sandberg and Moore, 1957 , Roberts *et al* 1958) Clumps of large atypical cells with large hyperchromatic nuclei in which one or more nucleoli can be seen contribute the classical picture In our cases whenever possible a tumour smear was made for comparison

Smears are classified as definitely positive and negative, but there remain a number which contain atypical hyperchromatic non-blood cells which cannot be definitely identified as malignant These we have classified as suspicious Fig 2–7 show examples of the types of cells we have seen

Results

Our complete results in all the 51 patients that we have investigated to date are given in Table I The 4 cases of mixed tumour of the parotid and the one case of benign gastric ulcer served as controls, and as expected showed no tumour cells in the blood It will be seen that tumour cells or suspicious cells were found in a significant proportion of all cases either in regional vein blood, or in peripheral vein blood, or in both The frequency varied in the different groups, and was highest in the regional vein blood in cases of tumours of the gastro-intestinal tract, though the figures are too small for final conclusions on this point

Further Analysis of the Pre- and Post-Hepatic Blood Studies

Fourteen patients had blood taken from the hepatic veins Five, a heterogeneous group including cases of secondary deposits in the liver from carcinoma of the breast, did not have portal vein blood taken , none of these 5 patients showed tumour cells in the hepatic vein blood Nine patients had samples taken both of pre-hepatic (i e portal) and of post-hepatic (i e hepatic vein) blood In 4 cases the portal blood was positive for malignant cells and in a further 4 suspicious, making a total of 8 out of 9 cases positive or suspicious The hepatic vein blood was positive in one case and suspicious in a further case, making a total of 2 out of 9 positive or suspicious One of the 9 patients, an advanced carcinoma of the

Table I —*Results of Examination of Regional, Peripheral and Hepatic Blood Samples for Tumour Cells*

		A Regional vein blood			B Peripheral (1) vein blood			C Hepatic vein blood			D Peripheral (2) vein blood		
	Number of cases	Pos	Susp	Pos + Susp	Pos	Susp	Pos + Susp	Pos	Susp	Pos + Susp	Pos	Susp	Pos + Susp
Breast	19	3/15	3/15	6/15	3/10	2/10	5/10	0/2	—	—	—	—	—
Parotid	4	2/4	0/4	2/4	—	—	—	—	—	—	—	—	—
Mixed parotid tumour	4	0/4	0/4	0/4	—	—	—	—	—	—	—	—	—
Gastro intestinal Ca	17	8/15	4/15	12/15	1/11	2/11	3/11	1/14	1/14	2/14	1/9	2/9	3/9
Miscellaneous Ca (thyroid bone, kidney lip melanoma)	6	2/4	1/4	3/4	0/3	1/3	1/3	—	—	—	—	—	—
Gastric ulcer	1	0/1	0/1	0/1									

Results expressed as No Pos or Susp No patients sampled
Total cases 51
Pos Positive for tumour cells
Susp Suspicious of tumour cells (hyperchromatic non blood cells)
Peripheral vein blood (1) = All cases in which peripheral vein samples were taken
Peripheral vein blood (2) = Cases which had in addition peripheral vein samples at time of portal samples

stomach with secondary deposits in the liver, showed large numbers of tumour cells in blood taken from all sites—portal vein, hepatic vein and peripheral venous blood, but they were much more numerous in the portal than in the hepatic blood

DISCUSSION

Our results confirm those of Engell (1955), Moore, Sandberg and Schubarg (1957), and Cole *et al* (1958) in showing that tumour cells can frequently be demonstrated in the blood in cases of malignant disease Though our figures are too small and the cases too heterogeneous for precision, both in site of growth, malignancy and degree of spread the entrance of tumour cells into the blood stream in malignant disease is clearly a frequent event, particularly since their demonstration represents the situation in a small sample at the moment of sampling only Blood borne metastasis, though frequent, is not as frequent as this, and most tumour cells which get into the blood stream must be destroyed The possibility exists that the attempted destruction of cells in the blood stream by chemotherapeutic agents might make matters worse by lowering the resistance of the normal tissues unless selective agents were discovered

The pre- and post-hepatic blood investigations show that the liver is a highly, but not completely, efficient filter of tumour cells reaching it by the portal vein, a fact already well known both from morbid anatomy and from experimental studies (Patey, 1937) If the one patient is excluded who had large numbers of malignant cells in all samples and who might be likened to an *in vivo* tissue culture of malignant cells, 7 out of 8 portal vein blood samples contained either tumour cells or suspicious cells, while only one of 8 samples of hepatic vein blood from the same patients was suspicious of tumour cells

Though again the figures are too small to be significant, it will be noted that the peripheral vein blood contained tumour cells or suspicious cells more frequently than the hepatic vein blood, both in the samples taken before operation (Table I, Column B) and in those taken at operation (Table I, Column D) If this finding were confirmed, it would suggest either that tumour cells are released from the liver sporadically and that the finding of them in the hepatic vein depended on the chances of timing, or that the cells enter the systemic blood stream by another route An obvious alternative route would be through lymphatics and the thoracic duct

---

EXPLANATION OF PLATE

FIG 2 —Seven tumour cells from a patient with carcinoma of the stomach Also several peroxidase positive neutrophils as well as erythrocytes and lymphocytes

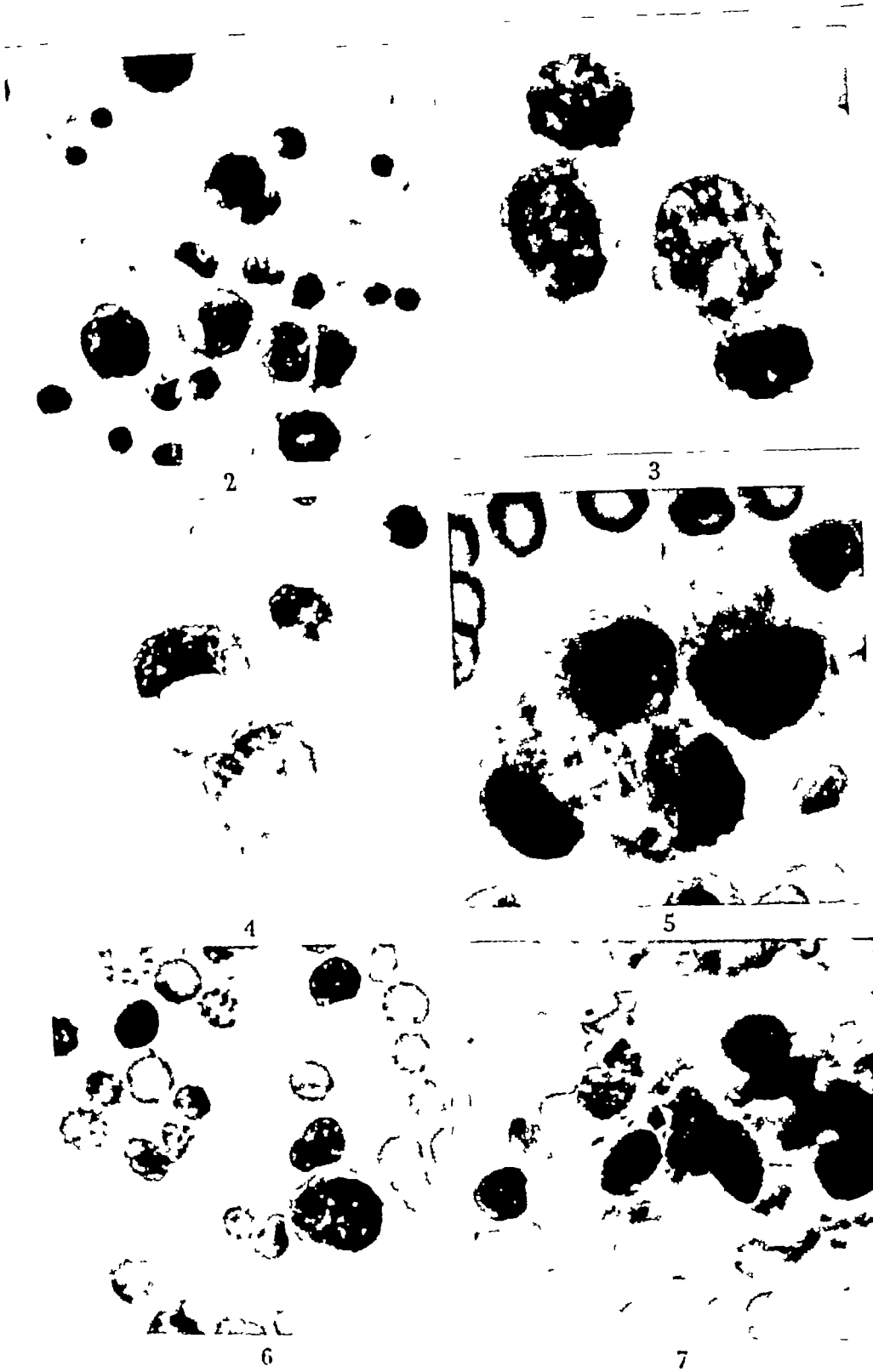
FIG 3 —Two malignant cells from the same patient with a peroxidase positive neutrophil near by for comparison

FIG 4 — Three tumour cells, one of which is a typical signet ring cell from the same patient as Fig 2 and 3

FIG 5 —A large clump of tumour cells from the external jugular vein of a patient with carcinoma of the parotid

FIG 6 —An atypical hyperchromatic non blood cell

FIG 7 —A rolled up sheet of endothelial cells scraped from a vein at autopsy Note pale staining, and, although poorly seen in reproduction, the very pale cytoplasm holding the cells together



2 3 4 5 6 7

SUMMARY

1 Tumour cells or suspicious cells were found in a significant proportion of 51 cases, both in blood draining malignant tumour sites and in peripheral venous blood

2 Tumour cells or suspicious cells were found in the portal blood in 8 out of 9 cases of carcinoma of the gastro-intestinal tract, but in only 2 of the same cases in hepatic vein blood

3 There is a suggestion that some tumour cells from carcinomas of the gastro-intestinal tract may reach the systemic circulation by the lymphatic route

The authors wish to express their sincere thanks to Mr D H Patey for the concept of pre- and post-hepatic studies, permission to study the above patients and for his very helpful suggestions regarding the text of the article. In addition we wish to thank the surgeons of the Department of Surgical Studies for the taking of blood samples and Dr J N Pattinson for radiological guidance in the performance of hepatic catheterization One of us (W S F) is in receipt of a grant from the American Cancer Society, and part of the expense of this investigation was defrayed by the British Empire Cancer Campaign

REFERENCES

Cole, W H , Roberts, S , Watne, A , McDonald, G and McGrath, E —(1958) *Bull N Y Acad Med* , **34**, 163

Engell, H C —(1955) *Acta chir scand* , Suppl 201

Fawcett, D W and Valle, B L (1952) *J Lab clin Med* , **39**, 354

Moore, G E , Sandberg, A A and Schubarg, J —(1957) *Amer J Surg* , **146**, 580

Patey, D H —(1937) *Brit J Surg* , **24**, 780

Roberts, S W A , McGrath, R , McGrew, E and Cole, W H —(1958) *Arch Surg* , *Chicago*, **76**, 334

Sandberg, A A and Moore, G E —(1957) *J nat Cancer Inst* , **19**, 1

Idem, White, L C and Schubarg, J —(1958) *Cancer*, **11**, 1180

RETICULIN PATTERNS IN TUMOURS OF LYMPHOID TISSUE

D H MACKENZIE

From the Department of Morbid Anatomy, Westminster Hospital, London

Received for publication January 23, 1959

In a previous communication (Mackenzie 1958) the diagnostic value of reticulin patterns in carcinomas and sarcomas was discussed Lesions falling under the general heading of tumours of lymphoid tissue were not included The purpose of the present article is to inquire into the diagnostic value, if any, of a study of the reticulin content and patterns in these lesions As before, reticulin refers to the argyrophilic fibres present within the neoplasm

Any inquiry into diseases of lymphoid tissue encounters the major difficulty of terminology Ignorance of aetiology and profound differences of opinion regarding the neoplastic nature of certain lesions have resulted in a complex nomenclature Not only do the well-recognised conditions have a variety of names but certain rarer ones, such as reticular lymphoma, are not considered as definite separate entities by some authorities In addition, the differentiation of such a lesion from Hodgkin's disease depends on a number of distinctions so fine that, in some cases, no unanimity of opinion would be obtained on a given section amongst a group of observers—however experienced Thus, in discussing reticulin patterns, it is only possible to describe the distribution of argyrophilic fibres in typical examples of conditions generally accepted as entities The terminology used here is that of Lumb (1954) It has the advantage of being simple and, in the present state of our knowledge, loses nothing by that simplicity

The normal lymph-node

In order to be able to assess abnormal reticulin patterns in lymph-nodes, a knowledge of the normal range of variability is essential As Marshall (1956) has rightly pointed out, the so-called " normal " lymph-node is exposed to a wide variety of stimuli and consequently shows a wide degree of morphological variation Thus, any description of a normal node refers to some extent to an abstract and idealised structure In addition to text books on histology, the anatomy of lymph-nodes has been described by Robb-Smith (1938) and Marshall (1956) Robb-Smith pointed out that, within a normal gland, only three structures differed histologically (1) the sinuses , (2) the follicles , and (3) the reticular tissue, wherever it lay in the gland Reticulin fibres penetrate all parts They form a heavy network on the inner surface of the capsule, on the trabeculae which pass inwards from the capsule and around the adventitia of the arteries and veins They are fairly plentiful throughout the reticular tissue but are less prominent in the sinuses Reticulin fibrils are seen within both true and pseudo follicles though, as a rule, they are scantier in the former type An anatomical diagram of a normal lymph-node is shown in Fig 1

Through the years, a number of writers have commented upon reticulin patterns in lesions of lymphoid tissue and references are given in each section In the present

series 305 cases were studied and these are considered under the headings shown in Table I The sections were stained by haematoxylin and eosin and by a slightly modified version of Gomori's silver impregnation technique

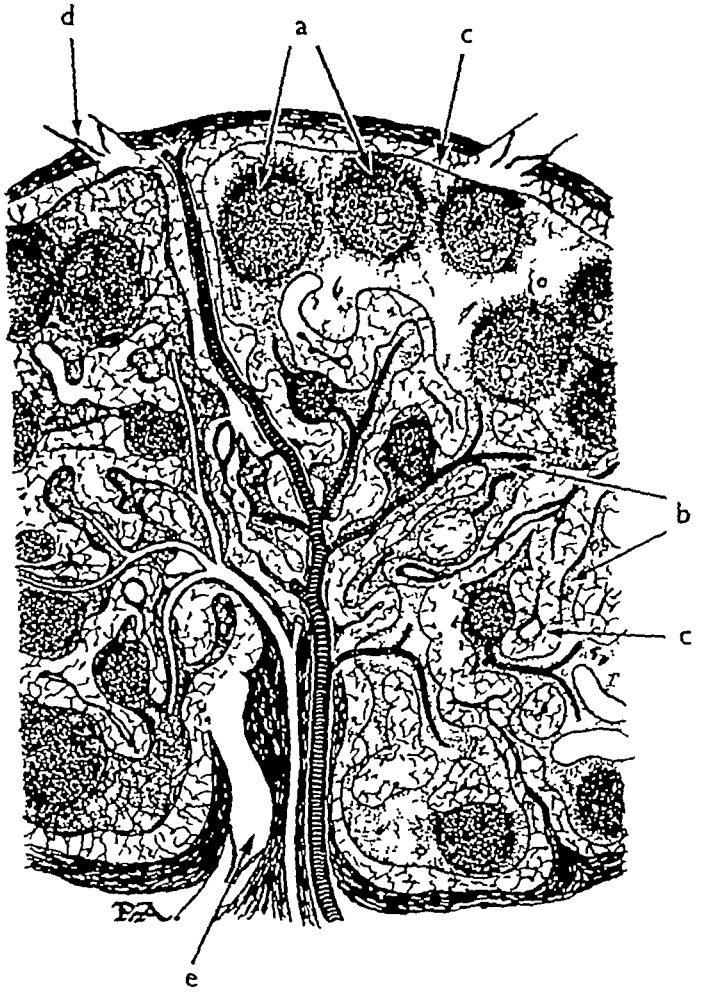


FIG 1 —Anatomical diagram of normal lymph node (modified after Heudorfer (1921) *Z Anat u Entwickl* 61, 365)

(*a*) Primary follicles (*b*) Medullary pulp (*c*) Sinuses (*d*) Afferent lymphatic (*e*) Efferent lymphatic

The reticulin framework of the node is shown on the left side of the diagram

TABLE I

Type of tumour	Number of cases
Hodgkin's disease	147
Lymphosarcoma	65
Anaplastic sarcoma of lymphoid tissue	35
Reticulum cell sarcoma	27
Follicular lymphoma	21
Reticular lymphoma	10
Total	305

Hodgkin's disease (lymphadenoma, Hodgkin's granuloma, fibromyeloid medullary reticulosis)

Hodgkin's disease is the commonest of the tumours of lymphoid tissue and the reticulin content has been described in many communications Pullinger (1932), Jackson and Parker (1947), Robb-Smith (1947), Lumb (1954) and Marshall (1956) have all commented on the reticulin increase which is nearly always present Robb-Smith (1938) noted that, occasionally, there is collagenous increase without a corresponding increase in reticulin Harrison (1953) noted scanty reticulin in certain rapidly growing lesions, while Lumb (1954) states that an increase in the argyrophil network precedes the laying down of collagen

The present series contained 147 cases Eighty showed a gross increase in the reticulin content of the gland (Fig 2) In 62 cases the reticulin was unmistakably increased, though to a lesser degree Thus 96 per cent showed some reticulin increase In only five cases of undoubted Hodgkin's disease was the reticulin content approximately within normal limits as regards quantity In all cases studied the distribution was abnormal and the gland architecture was destroyed There was no precise correlation between the cellularity of the disease and the reticulin content, although the reticulin tended to be less in the rapidly growing lesions Occasionally glands showed dense collagen formation while the remaining cellular areas contained only a few reticulin fibrils (Fig 3)

Lymphosarcoma (lymphocytoma and lymphoblastoma)

This term is widely accepted to describe a lymphoid tumour made up of lymphocytes or lymphoblasts or a mixture of the two Comments on the reticulin content have been made by Robb-Smith (1938), Jackson and Parker (1947), Dukes and Bussey (1947), Harrison (1953), Lumb (1954) and Evans (1956) There has been universal agreement that the reticulin is seldom increased and often shows a decrease

There were 65 cases in the present series In 33 cases there was a considerable reduction in the total reticulin content while in 27 cases the reduction was less apparent Thus 92 per cent showed some decrease The most typical picture showed destruction of gland architecture with reticulin fibrils of varying size scattered, often at wide intervals, between the tumour cells (Fig 4) Lymphosarcomas developing from follicular lymphomas often showed a remnant of follicular pattern In 5 untreated cases of lymphosarcoma the reticulin was increased (Fig 5)

EXPLANATION OF PLATES

FIG 2 —Hodgkin's disease Greatly increased reticulin Silver impregnation × 95

FIG 3 —Hodgkin's disease (sclerotic type) Silver impregnation × 25

FIG 4 —Lymphosarcoma Diminished reticulin Silver impregnation ×165

FIG 5 —Lymphosarcoma This amount of reticulin might well be seen in a reticulum cell sarcoma Silver impregnation × 110

FIG 6 —Reticulum cell sarcoma Reticulin not greatly increased Silver impregnation × 110

FIG 7 —Reticulum cell sarcoma Greatly increased reticulin Silver impregnation × 165

FIG 8 —Follicular lymphoma Condensation of reticulin around follicles Silver impregnation × 55

British Journal of Cancer

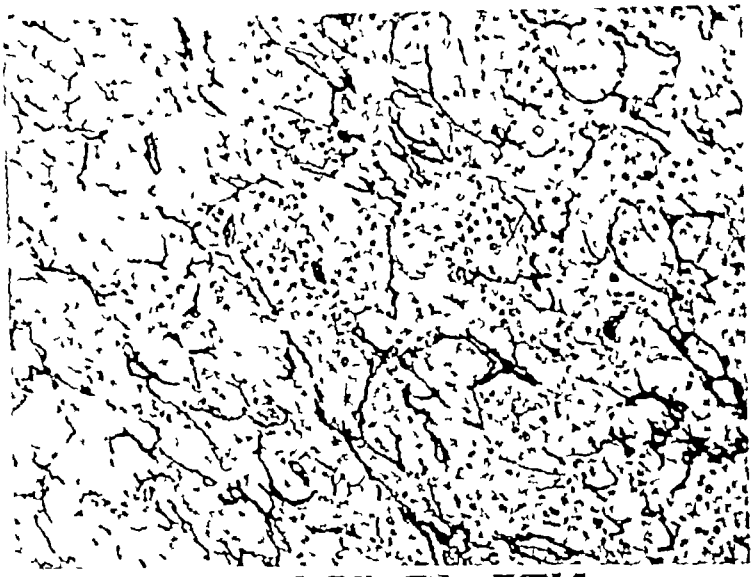
2

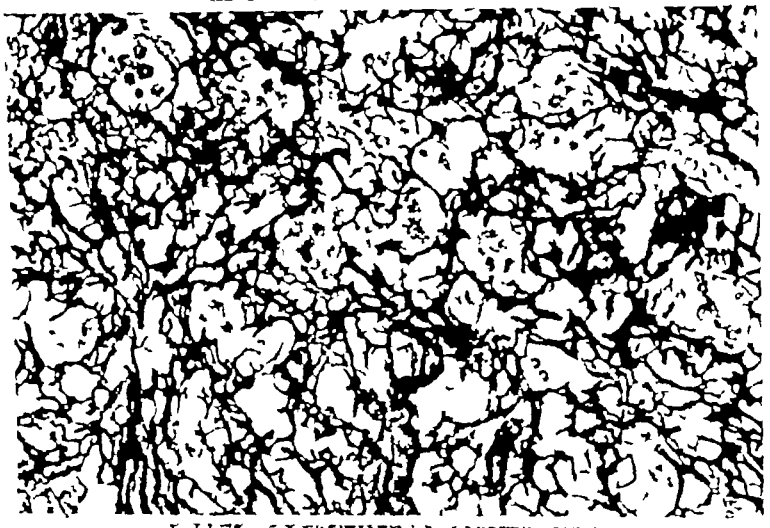
3

4

5

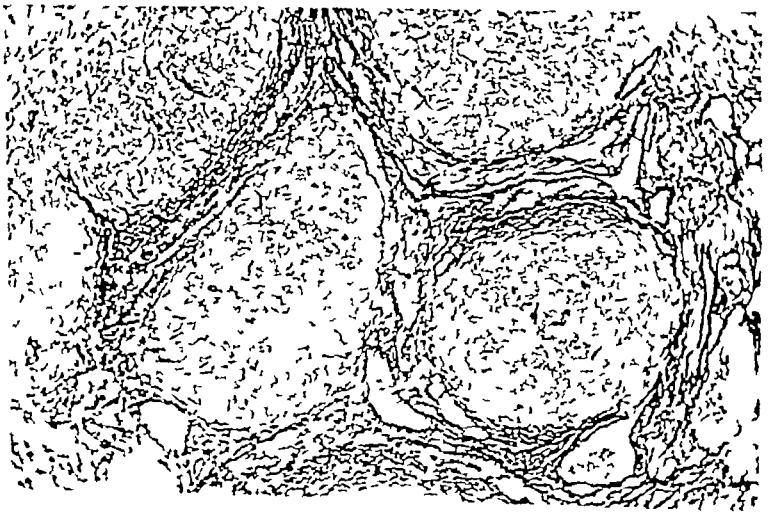
6





7

8



Anaplastic sarcoma of lymphoid tissue (Hodgkin's sarcoma, lymphoblastic reticulosarcoma, stem cell sarcoma and others)

This group includes the anaplastic variants of the other types The reasons for collecting these anaplastic and pleomorphic tumours into a simple group have been given by Lumb (1954) The principal cells involved are immature forms of the lymphocyte and reticulum cell and giant forms, multi-nucleated cells and mitotic figures are often seen in large numbers Under various names these tumours and their reticulin contents have been discussed by Callender (1934), Robb-Smith (1938), Jackson and Parker (1947), Harrison (1953), Evans (1956) and others

There were 35 cases in the present series With such a wide variation in the cytology it was not surprising to find an equally wide variation in the reticulin pattern and content In addition the reticulin density often varied considerably in different parts of a gland Areas showing only a few scattered fibrils lay next to other fields where there was a dense network of reticulin Sclerosis and collagen formation were also seen The variability of reticulin pattern within a gland often made an assessment of the total content difficult However in 21 cases there appeared to be a considerable overall increase in reticulin while 11 showed a more moderate one In two very cellular tumours the reticulin was decreased

Reticulum cell sarcoma [reticulo-sarcoma, lymphosarcoma (reticulum cell type) and others]

The difficulty of terminology is particularly apparent in any discussion of reticulum cell sarcoma In this context the term is used in the manner of Warren and Picena (1941) and Lumb (1954) It refers to a tumour made up of malignant reticulum cells which, though they may show some pleomorphism, are yet sufficiently uniform in type for the lesion to be distinguished from the anaplastic sarcomas Harrison (1953) described a similar lesion under the title reticulosarcoma Other writers who have described the reticulin patterns of lesions made up predominantly of reticulum cells include Callender (1943), Robb-Smith (1938) and Jackson and Parker (1947)

Twenty-seven cases were studied In 16 cases there was a marked increase in reticulin Eight cases showed a more moderate increase while in 3 glands the reticulin content appeared approximately within normal limits Thus 89 per cent showed some reticulin increase though the range was wide (Fig 6, 7) In all cases except one, silver impregnation showed complete destruction of the gland architecture In the one case only part of an enlarged gland was involved and the contrasting reticulin patterns of the normal and neoplastic areas were clearly seen

The reticulin patterns were of the sarcomatous fibrillary type and the reticulin density was often constant throughout a gland The fibrils were usually fine and scattered diffusely amongst the cells Sometimes individual prolongations joined one cell with another and occasionally single cells were surrounded by a sheath of reticulin Sclerosis and collagen formation were not observed

Follicular lymphoma (giant follicular lymphoblastoma, lymphoid follicular reticulosis and Brill-Symmer disease)

This is another lesion to which several names have been given The first detailed accounts were those of Brill, Baehr and Rosenthal (1925) and Symmers

(1938) Other communications on the subject have been those of Symmers (1942), Baggenstross and Heck (1940), Gall and Mallory (1942), Robb-Smith (1938 and 1947), Wetherby-Muir, Smith and Anderson (1952), Harrison (1953) and Lumb (1954) These writers noted that silver impregnation clearly emphasised the follicular pattern and that there was often a condensation of reticulin round the periphery of the follicles Within the follicles the reticulin was stated to be scanty or absent Rappaport, Winter and Hicks (1956) have subdivided follicular lymphoma into five types and noted variations in the reticulin patterns

There were 21 cases in the present series The pattern was quite different from that of the other lesions described Follicular structures were present throughout the substance of the glands and there was often severe compression of the sinuses and medullary tissue Silver impregnation clearly demonstrated these structures which were not always clearly apparent in sections stained with haematoxylin and eosin Within the follicles themselves there were often a number of tiny fibrils while a dense condensation of reticulin round the periphery was a common finding and was seen in 13 cases (Fig 8) The writer is in agreement with Rappaport that this condensation is seen when the follicles are closely packed and may also occur in reactionary hyperplasia When sarcomatous change supervenes in follicular lymphoma a break occurs in the ring of peripheral reticulin and the follicles become increasingly ill-defined Eventually, even with silver impregnation, they are no longer discernible and the reticulin pattern becomes that of the more malignant form, often a lymphosarcoma

Reticular lymphoma (*Hodgkin's paragranuloma and lymphoreticular medullary reticulosis*)

This is the rarest of the lymphoid tumours and its existence as an entity entirely separate from Hodgkin's disease is doubted by some authorities (Marshall, 1956) The reticulin content which most workers have found to be within normal limits or slightly increased has been described by Jackson (1937), Jackson and Parker (1944*a*, *b*, 1947), Robb-Smith (1947), Harrison (1952) and Marshall (1956) Harrison drew particular attention to the lobulation which was present in all his cases The glands were divided into large lobules by collagen and subdivided into smaller lobules by reticulin

Ten cases were available for study The gland architecture was destroyed in all cases and in five of them the lobulation described by Harrison was observed In these cases the intra-lobular reticulin was relatively scanty and made up of scattered short fibrils of varying thickness In the other five cases scattered fibrils were seen throughout the glands In amount the reticulin was considerably less than that observed in the vast majority of cases of Hodgkin's disease and somewhat greater than in typical lymphosarcoma

DISCUSSION

The reticulin in tumours of lymphoid tissue can be studied from the points of view of distribution and amount Harrison (1958, personal communication) stresses the great importance of using a silver impregnation method which clearly defines the argyrophilic fibres and does not allow nuclear staining to mask the smaller details He also draws attention to the information which can be gained

by use of a very low power, such as a × 10, hand lens in the initial examination of a section

The distribution of reticulin

A study of the reticulin pattern is of more value than any assessment of the amount present A low power view will show whether the basic structure of the gland architecture is preserved or not The number, size and distribution of any follicles present can be clearly seen and their relationships to the sinuses determined Although wide variations in pattern frequently occur, certain distributions are characteristic In Hodgkin's disease and in the anaplastic sarcomas the reticulin density is usually uneven, and dense masses of reticulin may lie adjacent to areas containing only a few fibrils To a lesser extent this is true of reticular lymphoma Lymphosarcomas and reticulum cell sarcomas often show a fairly uniform pattern In follicular lymphoma the reticulin distribution is quite unlike any other lymphoid tumour Unfortunately the pattern may closely resemble that seen in gross reactionary hyperplasia and is of little use in distinguishing these two conditions Apart from the primary lymphoid tumours, silver impregnation will often reveal the site of abnormal cells For example, Robb-Smith (1947) points out that, occasionally, in children with sinus catarrh of lymph-nodes, the histiocytes become multinucleated and this giant cell sinus reticulosis may well suggest a malignant process Silver impregnation reveals that these multinucleated cells are limited to the sinuses and follow up studies have suggested that this is a benign condition Similarly, a metastasis can often be distinguished from a primary lymphoid neoplasm With early invasion the tumour cells are confined to the sinuses while, in later cases, the invading tumour will possess its own reticulin pattern and will often be identifiable as a carcinoma

The amount of reticulin

Marked increases or decreases in the amount of reticulin in a lesion are easily observed Great caution is necessary before any importance is attached to minor changes The reticulin content of a tumour is often variable from one part to another and the overlap between a number of tumours in this group is considerable For example a reticulum cell sarcoma with a minimal reticulin content for this type of lesion, a reticular lymphoma or a lymphosarcoma with more than average reticulin may be virtually indistinguishable by silver impregnation methods Thus, while an increase of reticulin may help to confirm a diagnosis of reticulum cell sarcoma, a decrease does not exclude it It must also be remembered that the predominating cell type and the general histological picture may change as the disease progresses This has been discussed by Custer and Bernhard (1948) and other writers Such a change may be accompanied by a change in the reticulin content The most that a study of the gross amount of reticulin can do is to provide confirmatory evidence for a given diagnosis or, occasionally, to suggest an alternative one

SUMMARY

1 The reticulum content and pattern in 305 cases of tumours of lymphoid tissue have been examined

2 The definite but limited value of silver impregnation in the diagnosis of these tumours has been discussed

I wish to thank Sir Stanford Cade for permission to study many cases under his care I am indebted to Lt -Col P D Stewart, R A M C , and W/Cdr R M Cross, R A F , for the loan of blocks , to Springer-Verlag for permission to reproduce Fig 1 and to the Department of Medical Photography, Westminster Hospital

REFERENCES

BAGGENSTROSS, A H AND HECK, F J —(1940) *Amer J med Sci* , **200**, 17
BRILL, N E , BAEHR, G AND ROSENTHAL, N —(1925) *J Amer med Ass* , **84**, 668
CALLENDER, G R —(1934) *Amer J Path* , **10**, 443
CUSTER, R P AND BERNHARD, W G —(1948) *Amer J med Sci* , **216**, 625
DUKES, C E AND BUSSEY, H J R —(1947) *Brit J Cancer*, **1**, 30
EVANS, WINSTON R —(1956) ' Histological Appearances of Tumours ', London (Livingstone)
GALL, E A AND MALLORY, T B —(1942) *Amer J Path* , **18**, 381
HARRISON, C V —(1952) *J Path Bact* , **64**, 513 —(1953), ' Recent Advances in Pathology' 6th Edition, London (Churchill)
JACKSON, H —(1937) *Surg Gynec Obstet* , **64**, 465
Idem AND PARKER, F —(1944*a*) *New Engl J Med* , **230**, 1 —(1944*b*) *Ibid* , **231**, 35 —(1947) ' Hodgkin's Disease and Allied Disorders ', New York (Oxford University Press)
LUMB, G D —(1954) ' Tumours of Lymphoid Tissue ', London (Livingstone)
MACKENZIE, D H —(1958) *Brit J Cancer*, **12**, 14
MARSHALL, A H E —(1956) ' An Outline of the Cytology and Pathology of the Reticular Tissue ', London (Oliver and Boyd)
PULLINGER, B D —(1932) ' Rose Research on Lymphadenoma ', Bristol (John Wright)
RAPPAPORT, R , WINTER, W J AND HICKS, E B —(1956) *Cancer*, **9**, 792
ROBB-SMITH, A H T —(1938) *J Path Bact* , **47**, 457 —(1947) ' Recent Advances in Clinical Pathology ', London (Churchill)
SYMMERS, D —(1938) *Arch Path* , **26**, 603 —(1942) *Ibid* , **34**, 385
WARREN, S AND PICENA, J P —(1941) *Amer J Path* , **17**, 385
WETHERBY-MUIR, G , SMITH, P AND ANDERSON, H J —(1952) *Quart J Med* , **21**, 327

HORMONE EXCRETION IN PROSTATIC CANCER
AN ATTEMPT TO CORRELATE URINARY HORMONE EXCRETION AND CLINICAL STATE

R D BULBROOK, L M FRANKS AND F C GREENWOOD

From the Clinico-pathological Laboratories, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W C 2

Received for publication January 30, 1959

In a previous paper the excretion of oestrogens, 17-oxosteroids and 17-oxogenic steroids by men with untreated prostatic cancer was compared with that of controls of the same age, and the effects of treatment by synthetic oestrogens or by castration were briefly described (Bulbrook, Franks and Greenwood, 1959) The present paper describes in detail the changes which occur within the first two years of treatment of prostatic cancer by castration or by the administration of synthetic oestrogens and attempts to correlate the clinical effects of such treatment with the changes in hormone excretion in eight patients

MATERIAL

Eight patients with prostatic cancer, (aged 66 to 80 years), were admitted to hospital and 24 hour urine specimens collected for three to fifteen days before treatment The patients were re-admitted to hospital at intervals during treatment for clinical evaluation and urine collections The clinical diagnosis was confirmed histologically in five cases and cytologically (prostatic smear) in the remaining four Treatment was by surgical castration (three patients) or by synthetic oestrogens (five patients) The clinical assessment was made without knowledge of the biochemical findings

CHEMICAL METHODS

Chemical methods used are as previously described (Bulbrook, Franks and Greenwood, 1959) The effects of synthetic oestrogen treatment on the urinary oestrogen excretion are given for oestrone and oestriol only since the oestradiol-17β fraction cannot be measured accurately in urine from these patients Intravenous diethyl stilboestrol phosphate also interferes with the measurement of oestrone and in one case where this compound was given only oestriol values are reported

RESULTS

A brief description of the clinical and biochemical findings is given for each patient The hormone excretion of the 8 patients is shown graphically in Fig 1 to 8 Periods of clinical tumour activity are shown by a black bar , periods of remission by a white bar

All periods of time have been taken from the day of operation or the start of treatment The mean hormone excretion in the pre-treatment period—the control level—has been used as the baseline for comparing subsequent hormone determinations during treatment Age refers to that at operation or at the start of treatment

a *Castration its effects on the clinical state and on the excretion of oestrone, oestradiol-17β, oestriol, 17-oxosteroids and 17-oxogenic steroids (Group of three patients)*

Patient No 1, *aged* 66 *years*, was admitted with incontinence and supra-pubic pain The prostate was clinically malignant and a prostatic smear contained malignant cells The serum acid phosphatase (formalin inactivated) was 3 0 units There were no skeletal metastases on X-ray examination After a control period of 9 days the patient was treated by castration and per-urethral resection The resected tissue contained areas of anaplastic carcinoma The changes in hormone excretion are shown in Fig 1 In the immediate post-operative period there was a marked rise in excretion of all three groups of steroids measured This phenomenon has been frequently observed to follow operation and is probably due to an adrenal cortical reaction to stress After this initial rise the amounts of excreted oestrogens and 17-oxosteroids fell progressively to pre-operative levels Oestrogens were not detected in the urine 14 weeks after operation, and the 17-oxosteroid excretion was very low Clinically there was a marked improvement with complete freedom from symptoms Over the period from 33 to 65 weeks after operation, oestrogen was again detectable in the urine increasing to pre-operative levels and accompanied by an increase in 17-oxosteroid secretion This period was characterised by the appearance and subsequent development of metastases in the pelvis and spine When the patient complained of pain, 72 weeks after castration, stilboestrol was given This was followed by rapid objective and subjective improvement and a second fall in oestrogen and 17-oxosteroid levels The excretion of 17-oxogenic steroids remained unchanged throughout the period of observation

Patient No 2, *aged* 70 *years*, was admitted complaining of urinary difficulty for 6 months Clinically the prostate was malignant and prostatic smears contained malignant cells (confirmed by later biopsy) The serum acid phosphatase was 1 5 units There were no skeletal metastases on X-ray examination The patient was castrated and the post-operative hormonal changes are shown in Fig 2

A transient rise in oestrogen excretion in the immediate post-operative period was followed by a fall to below the pre-operative levels Later specimens obtained during follow-up showed a return of excretion to pre-operative values The 17-oxosteroid levels showed no significant change at operation or in the following 46 weeks, whereas the 17-oxogenic steroids showed a slow fall after operation to low levels but with one exceptionally high value Clinically the operation resulted in the disappearance of urinary symptoms and an arrest in the tumour growth up to 44 weeks The re-appearance of mild urinary symptoms was noted some 32 weeks after the return of oestrogen excretion to pre-operative levels, and on examination the prostate was enlarged and hard This patient died 78 weeks after castration with left ventricular failure Histologically the prostate removed at autopsy showed many areas of growing tumour

Patient No 3, *aged* 70 *years*, had a retropubic prostatectomy when 63 years old Histologically there was an area of prostatic carcinoma in the tissue removed No treatment was given

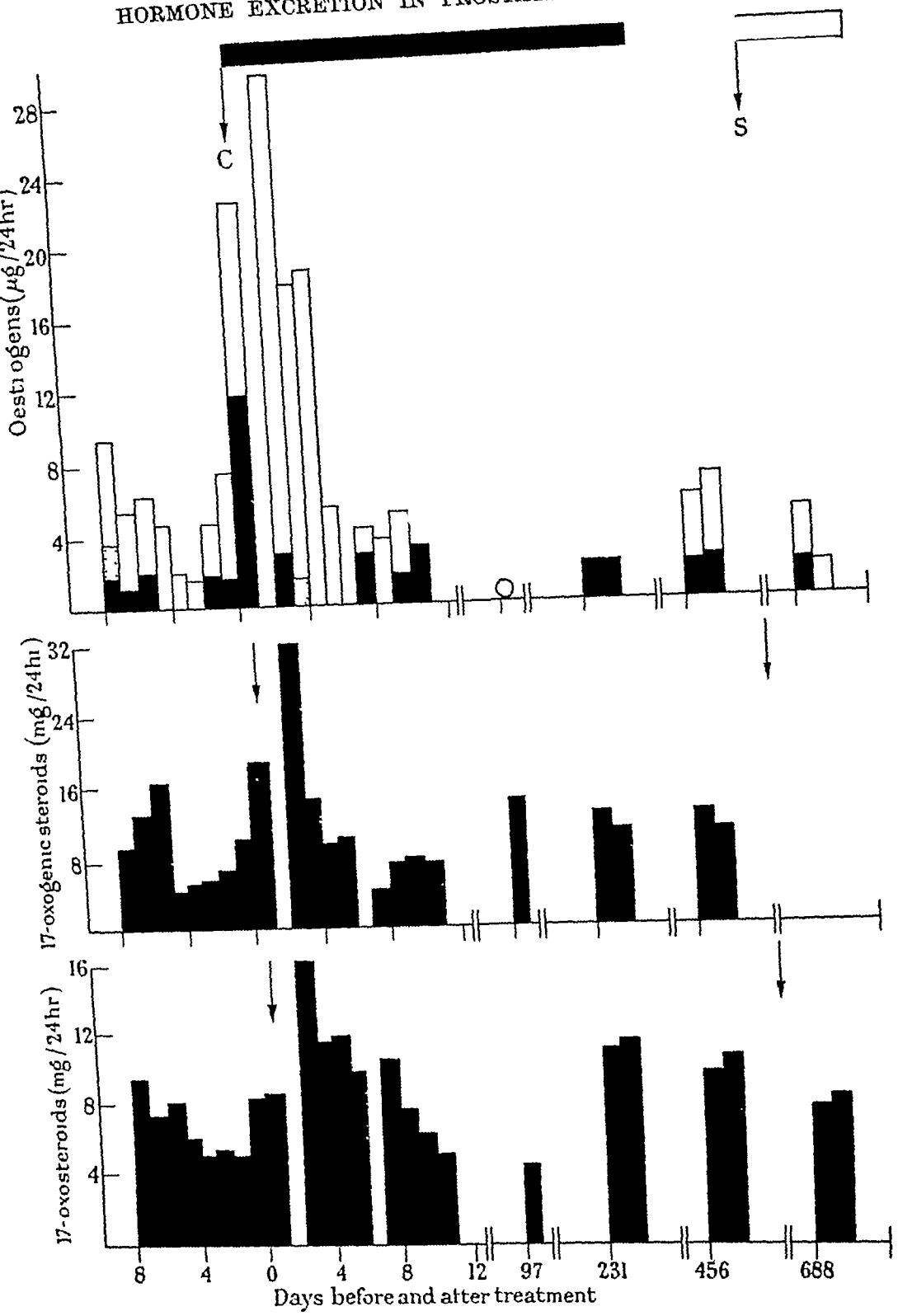


FIG 1.—The effect of castration on the excretion of oestrogens, 17 oxosteroids and 17 oxogenic steroids

Patient 1, aged 66 years

The vertical arrows show the day of treatment

C = castration
S = stilboestrol

Each vertical column gives the result of a duplicate determination on a 24 hour urine specimen. For the oestrogens the height of the block shows the total amount excreted, subdivided into oestrone (■), oestradiol (▭) and oestriol (□)

The horizontal bar at the top of the figure indicates clinical state. The black areas represent tumour growth, the white areas represent regression. All subsequent figures are plotted in the same way

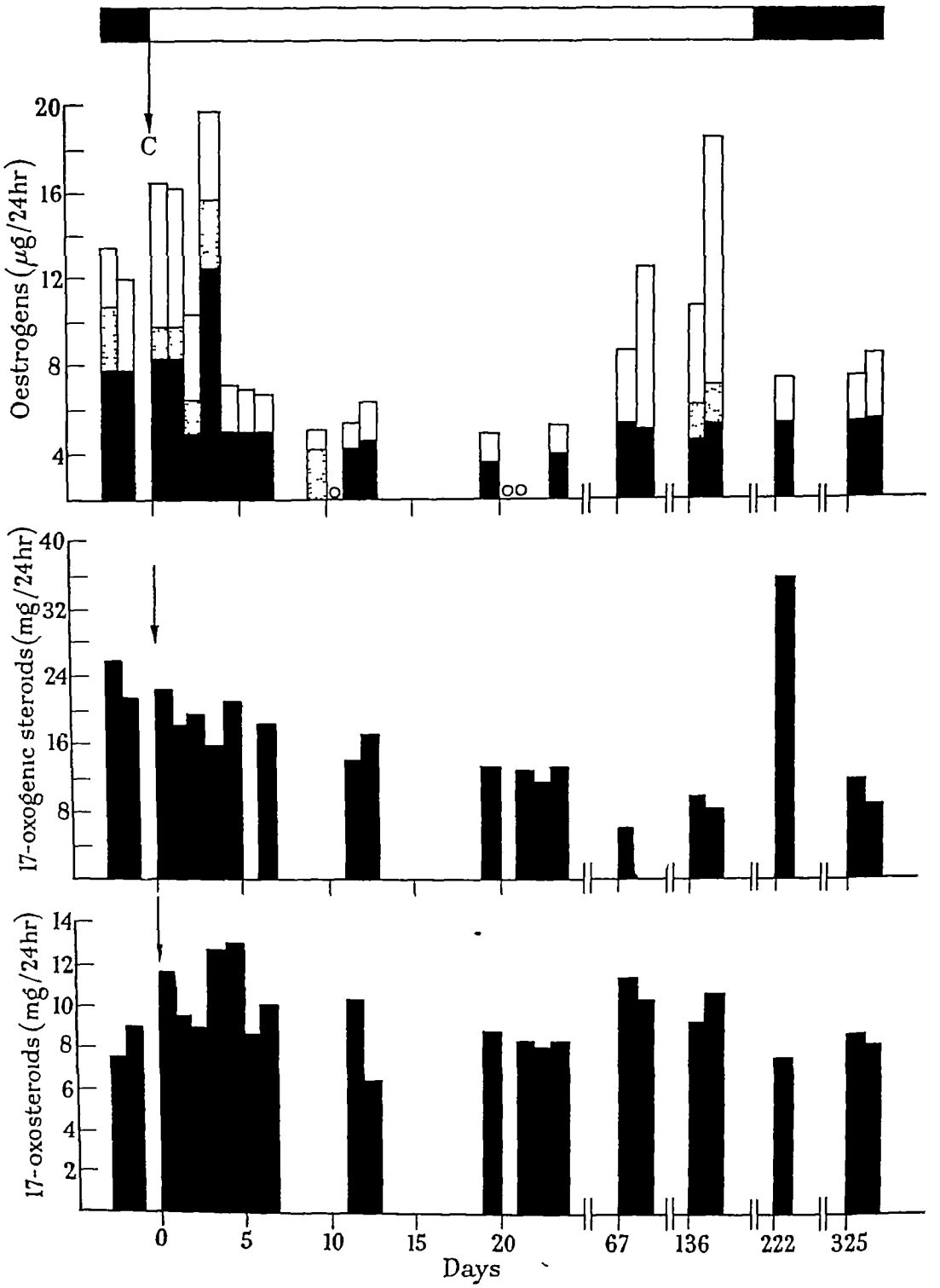


FIG 2.—The effect of castration on the excretion of oestrogens, 17 oxo and 17-oxogenic steroids

Patient 2, aged 70 years

He was admitted to hospital 7 years later with a two months' history of pain in the right thigh X-rays showed osteosclerotic deposits in the pelvis, femur and lumbar spine The serum acid phosphatase was raised (45 8 units) Castration resulted in clinical improvement, a fall in serum acid phosphatase to 2 3 units but no immediate reduction in urinary oestrogens, 17-oxosteroids or 17-oxogenic

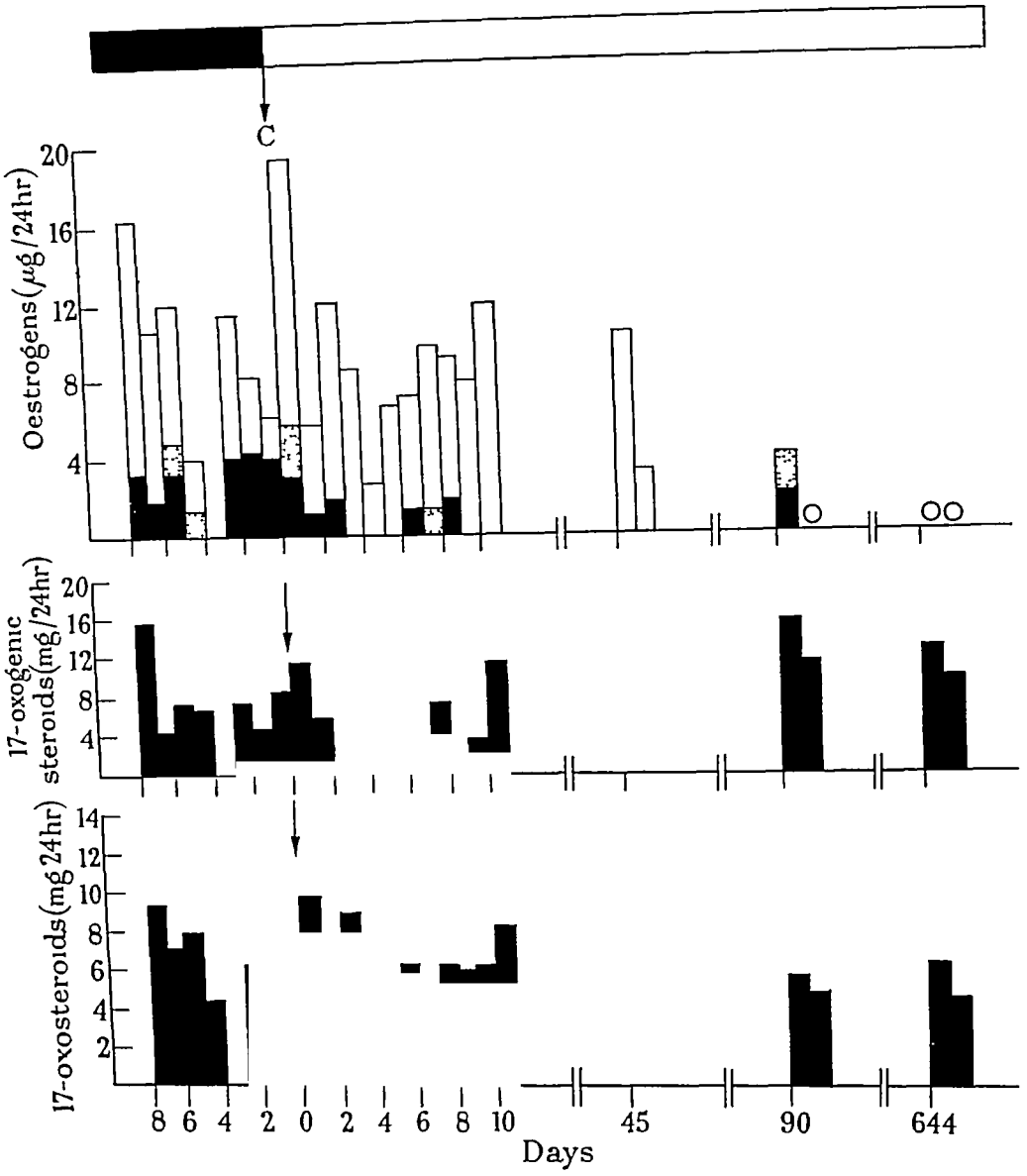


FIG 3 —The effect of castration on the excretion of oestrogens, 17 oxo- and 17 oxogenic steroids
Patient 3, aged 70 years

steroids Clinical improvement and the lowered serum acid phosphatase was maintained up to 92 weeks after operation During this time oestrogen excretion fell progressively to below pre-operative levels and at the last follow-up no urinary oestrogen was found, whilst the 17-oxosteroids and 17-oxogenic steroids have remained unchanged.

b *Synthetic oestrogen the effects of administration on clinical state and on the excretion of oestrone, oestriol, 17-oxosteroids and 17-oxogenic steroids (Group of five patients)*

Patient No 4, aged 69 years, when first seen complained of dysuria and backache, pain on micturition, suprapubic pain and left sciatic pain The prostate was moderately enlarged, fixed, hard and irregular A per-urethral resection was carried out Histological examination showed an adenocarcinoma with moderate fibroblastic reaction After a 6-day control period, stilboestrol was given (50 mg twice daily) There was little effect on oestrogen excretion for the first 5 days but thereafter the excretion of oestriol fell progressively (Fig 4) At the first follow-up

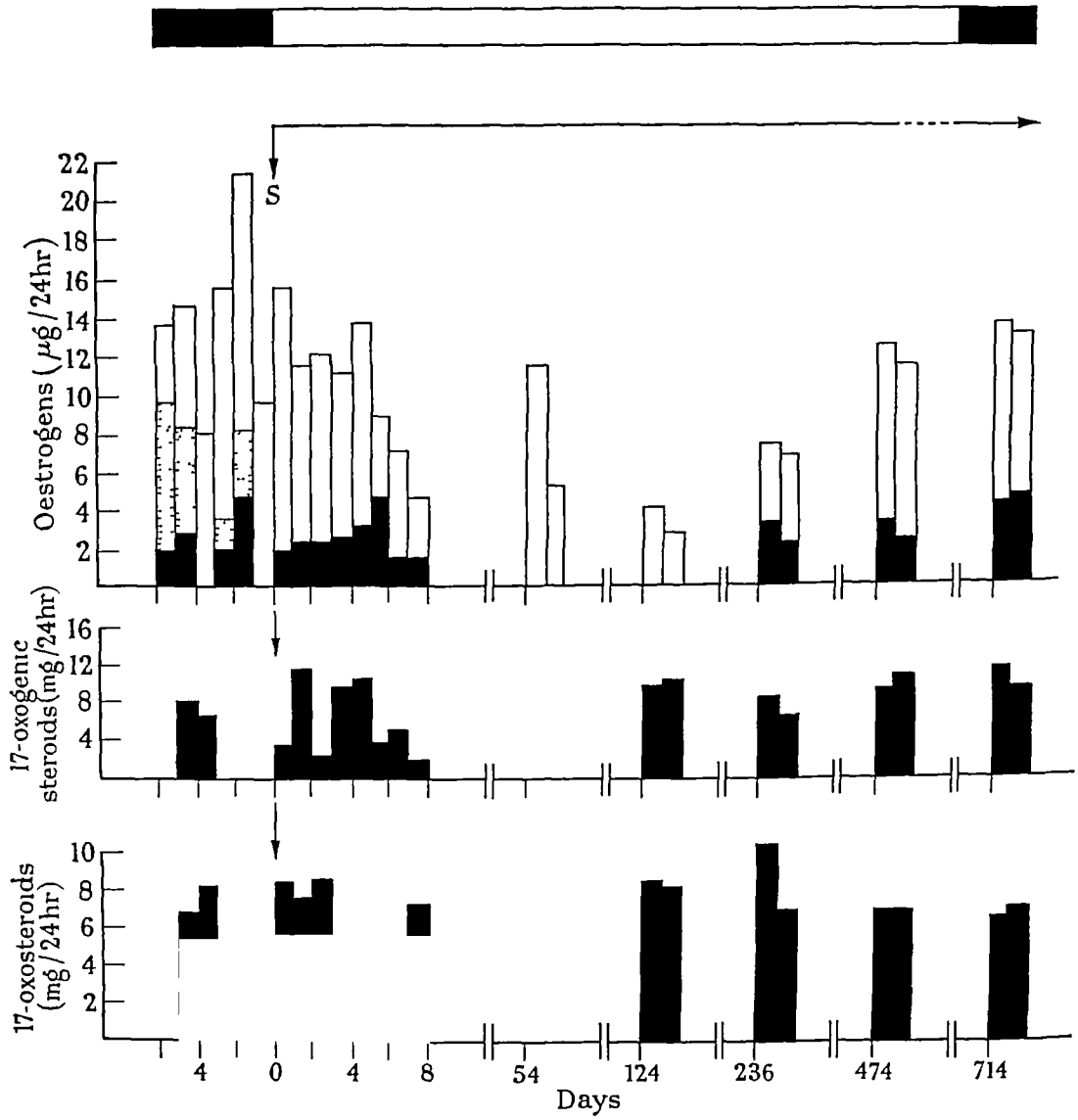


FIG 4 —The effect of synthetic oestrogen administration on the excretion of oestrogens, 17-oxo and 17 oxogenic steroids

Patient 4, aged 69 years

The dotted break in the line showing the period of stilboestrol treatment denotes a period when the patient stopped taking the drug

at 8 weeks, the amount of oestriol in the urine almost equalled the control level but after 18 weeks of treatment oestrogen levels were considerably depressed (30 per cent of control mean level) However, during three subsequent follow-up periods oestrogen excretion increased, and at 68 and 102 weeks the amounts were equivalent to the control levels For some of this time the patient had not taken stilboestrol regularly because of indigestion and subsequently dienoestrol was given (10 mg three times a day) The fall in endogenous oestrogen excretion after treatment was paralleled by clinical improvement, the rise to control levels was associated with a return of urinary symptoms

17-Oxosteroid and oxogenic steroid excretion was not altered by treatment The serum acid phosphatase before treatment was 2 5 units , at the last follow-up, at 102 weeks, it was 1 1 units

Patient No 5, *aged* 78 *years*, complained of severe frequency of micturition and dysuria The prostate was small and hard and a prostatic smear contained malignant cells There were no metastases radiologically After an 8-day control period, stilboestrol (50 mg twice daily) was given During the first few days of treatment both oestrogen and oxosteroid excretion rose but thereafter the amount of oestrogen excreted fell to a very low level, while 17-oxosteroids decreased slightly

After 8 weeks the stilboestrol was replaced by TACE (chlorotrianisene, 12 mg t d s) At the first follow-up, 13 weeks after the start of treatment, urinary oestrogens were still barely detectable and by this time 17-oxosteroid excretion was also greatly depressed At 37 weeks, oestrogen excretion had risen to control levels and 17-oxosteroids had increased slightly At 73 weeks, the amounts of 17-oxosteroids were approaching the mean control level The patient's clinical state improved on treatment and the improvement was maintained in spite of the rise in oestrogens and 17-ketosteroids Serum acid phosphatase levels fell from 7 5 before treatment to 0 4 units by the last follow-up 17-Oxogenic steroid levels were depressed, and remained so, at all follow-up periods

Patient No 6, *aged* 72 *years*, had suffered from backache and pain radiating down the left leg for 6 months The prostate was enormous and craggy, pressing on, but not ulcerating, the rectum The prostatic smear was positive The serum acid phosphatase was 120 units , the serum alkaline phosphatase, 92 units X-ray examination showed metastases in the lumbar and dorsal spine, pelvis, thoracic cage, and the upper end of the left femur After a 7-day control period the patient was treated by intravenous diethylstilboestrol phosphate for a period of 13 days without benefit The serum acid phosphatase at the end of this treatment was 122 units , the serum alkaline phosphatase was 82 units Administration of diethylstilboestrol phosphate made it impossible to measure oestrone and oestradiol-17β in the urine Oestriol levels were depressed by treatment 17-Oxosteroid excretion did not change but the level of 17-oxogenic steroids fell to approximately 30 per cent of control values Diethylstilboestrol phosphate was stopped and stilboestrol (50 mg b d) started Oestriol excretion declined further and 17-oxosteroid excretion, unaffected by the previous treatment, progressively fell , the 17-oxogenic steroids remained depressed The fall in hormone excretion was accompanied by an objective regression with decrease of pain The serum acid phosphatase fell to 75 units and the serum alkaline phosphatase fell to 43 units Over the following 20 weeks the serum acid phosphatase fell progressively to 15 units, the serum alkaline phosphatase to 18 units X-ray examination at 18 weeks showed that the metastases were more sclerotic

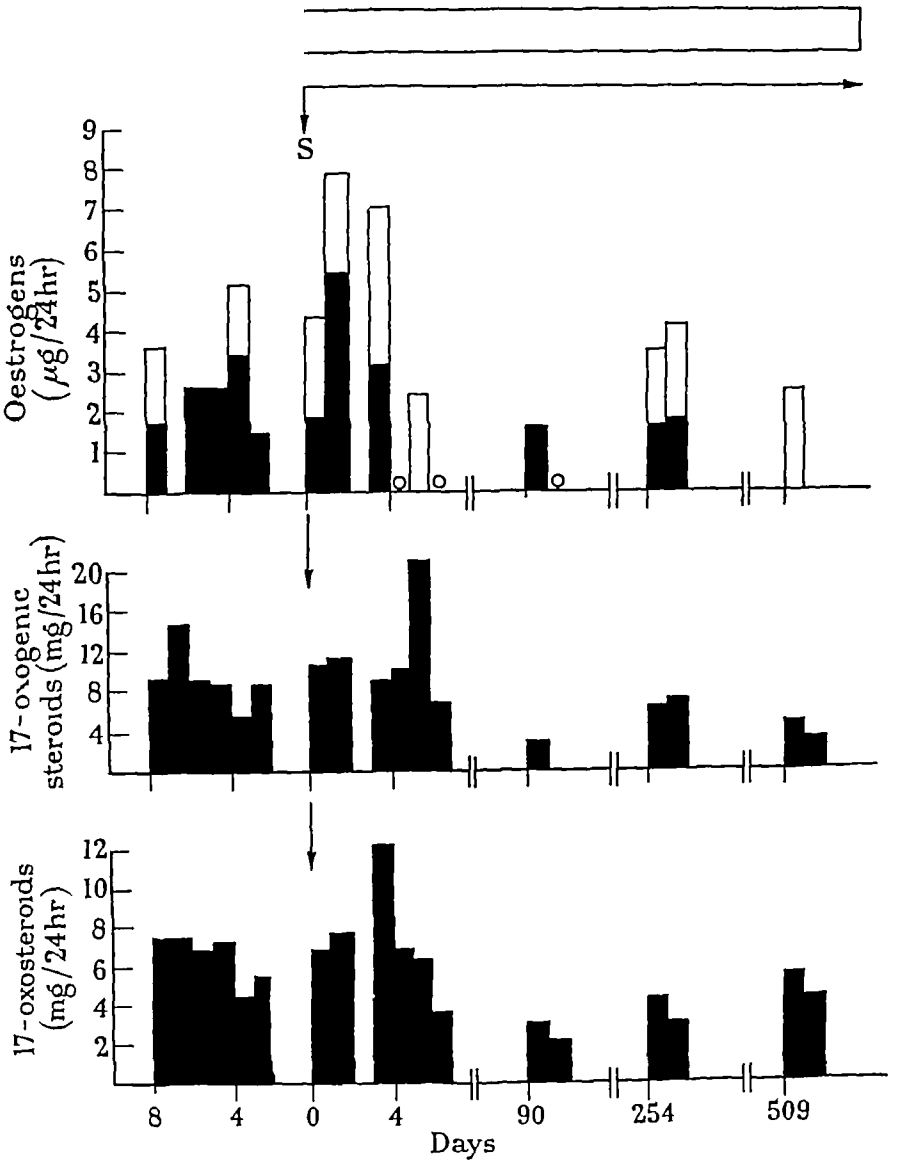


FIG 5.—The effect of synthetic oestrogen administration on the excretion of oestrogens, 17 oxo and 17 oxogenic steroids

Patient 5, aged 78 years

At the first follow-up (21 weeks) oestrogen levels were still low but 17-oxosteroids and 17-oxogenic steroids were at control levels X-ray examination showed metastases in the right shoulder The serum acid phosphatase was now 7 0 units and the serum alkaline phosphatase 12 units

At the last follow-up (54 weeks), oestrogen excretion had increased and, with the 17-oxosteroids, was at control levels, but 17-oxogenic steroids were depressed Although the general condition was good the secondary deposits previously noted had developed The serum acid phosphatase was 3 2 units and the serum alkaline phosphatase was 8 4 units

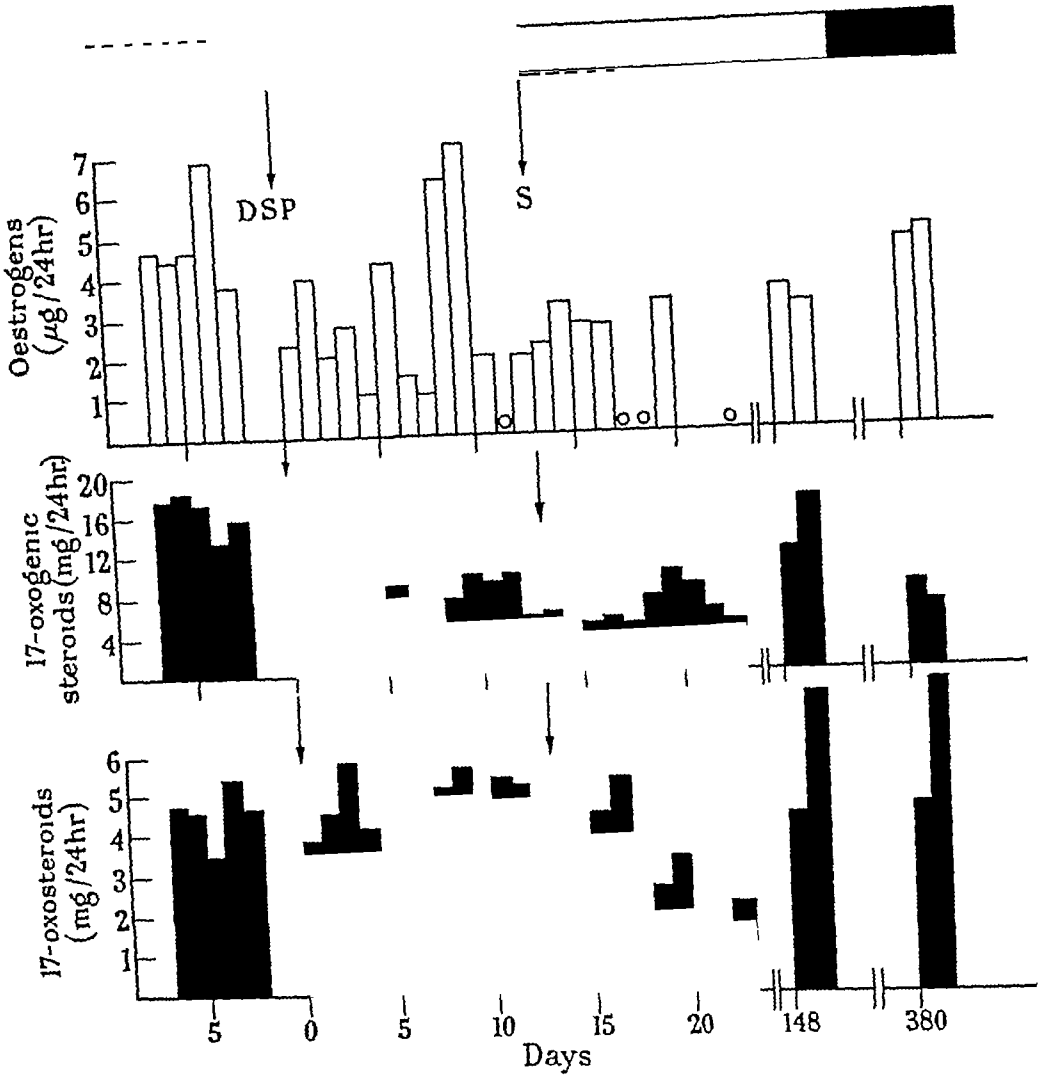


FIG 6.—The effect of synthetic oestrogen administration on the excretion of oestrogens, 17 oxo and 17 oxogenic steroids

Patient 6, aged 72 years

S = stilboestrol
D S P = diethylstilboestrol phosphate

Patient No 7, aged 80 years, was first admitted with acute retention of urine A per-urethral biopsy showed one small nodule of prostatic carcinoma of low grade Serum acid phosphatase was 1 1 units Fifteen months later there was haematuria and the prostate (per rectum) was hard and clinically malignant The urine deposit contained malignant cells TACE (12 mg t d s) was administered and over the first 10 days of treatment no change occurred in hormone excretion At the first follow-up (11 weeks) the patient was well At this stage, oestrogen excretion had fallen to zero , 17-oxosteroid and 17-oxogenic steroid levels were depressed Serum acid phosphatase was 1 5 units

At the second follow-up (34 weeks), oestrogen was again detected in the urine but at a level well below that of the control period 17-Oxosteroids and 17-oxogenic steroids were lower than at the previous follow-up The serum acid

phosphatase was 0 8 units The patient remained well but subsequently died of coronary thrombosis

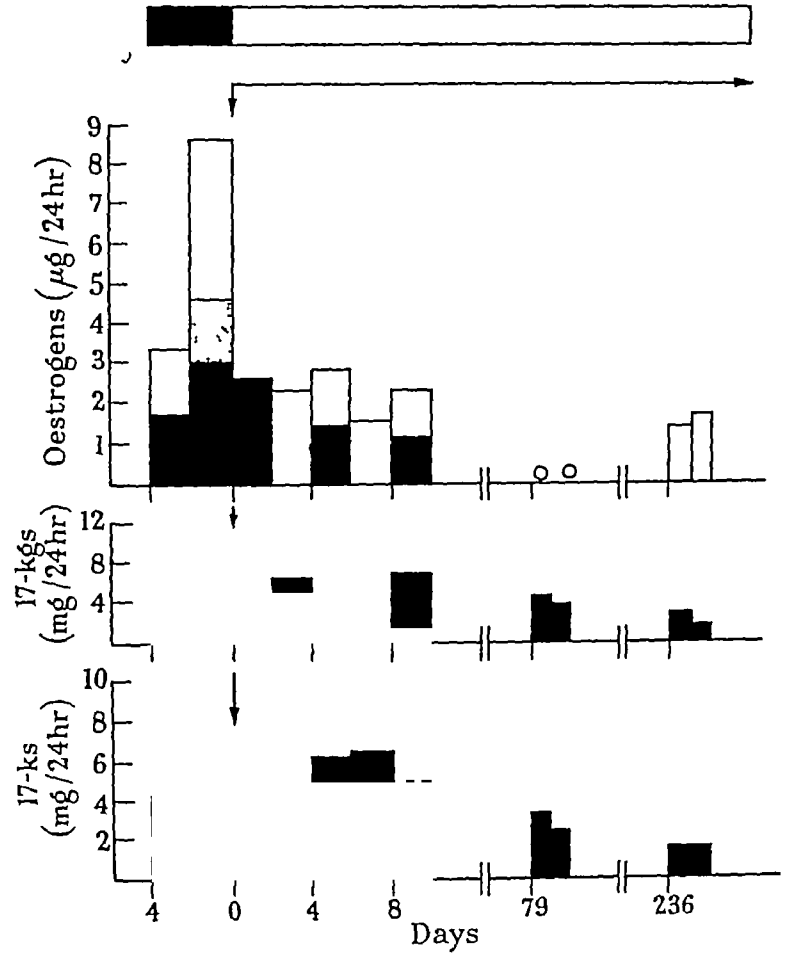


FIG 7 —The effect of synthetic oestrogen administration on the excretion of oestrogens, 17 oxo and 17 oxogenic steroids

Patient 7, aged 80 years

Patient No 8, *aged* 80 *years,* was admitted to hospital with slight frequency and pain on micturition On examination the prostate was hard and the smear positive The serum acid phosphatase was 0 4 units X-ray examination showed osteoarthritis in the spine but no secondary deposits Stilboestrol was administered (50 mg b d) after a 6-day control period Oestrogen excretion was very variable but in the first 8 days treatment did not affect the mean amount excreted 17-Oxosteroid excretion fell slowly over the 8 days studied , 17-oxogenic steroid excretion increased slightly

At the first follow-up (9 weeks) oestrogen excretion was zero and remained so at subsequent examinations 17-Oxosteroid excretion remained depressed throughout the 85-week period of study

At the third follow-up (27 weeks), 17-oxogenic steroid excretion was markedly depressed but rose to pre-treatment levels at 52 weeks and remained so at 85 weeks The serum acid phosphatase was normal throughout and the patient has remained in remission

The administration of synthetic oestrogens, therefore, generally resulted in a fall in the excretion of endogenous oestrogen either immediately or after a transient rise during the first few days of treatment The fall was not permanent since in only one case were the oestrogen levels permanently depressed

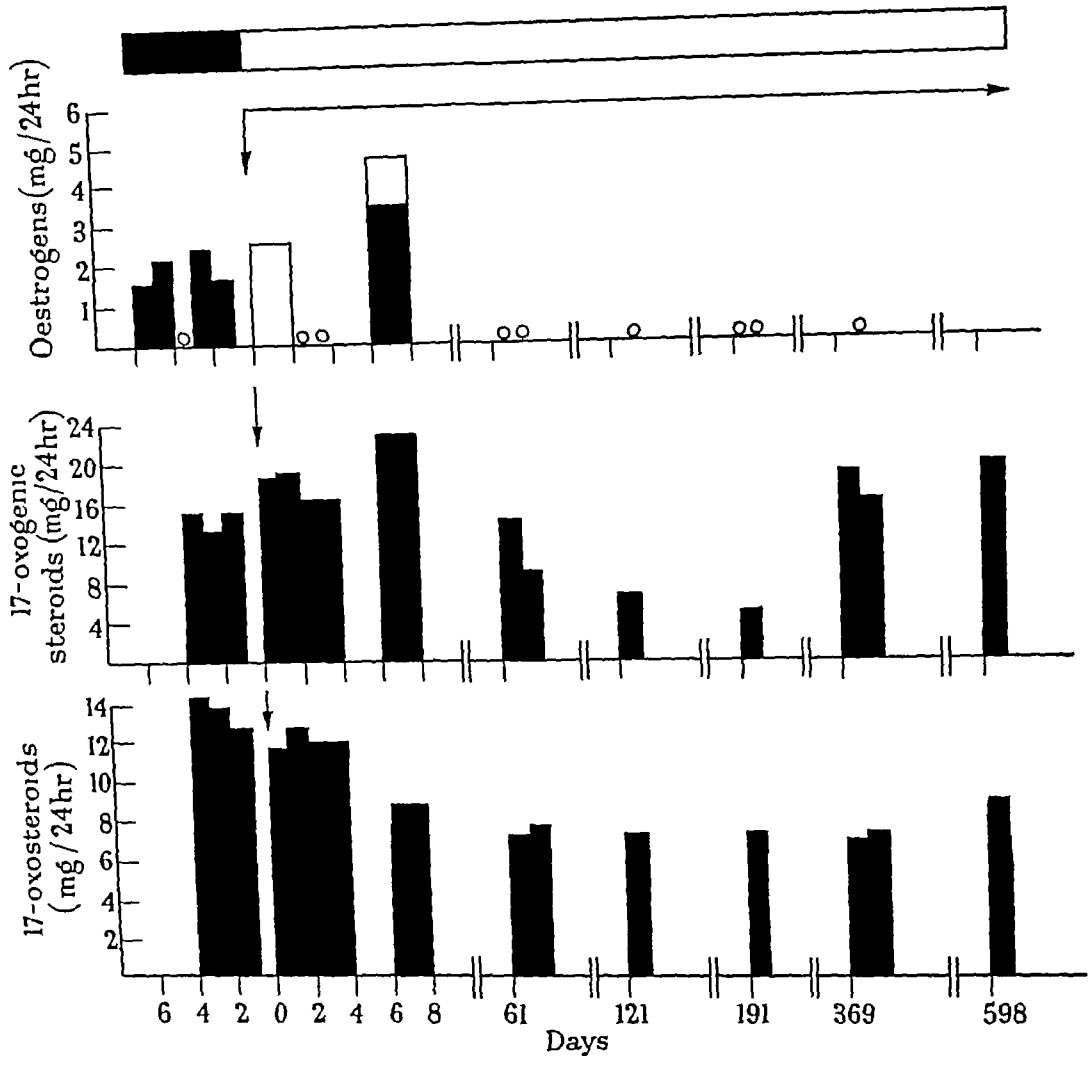


FIG 8 —The effect of synthetic oestrogen administration on the excretion of oestrogens, 17 oxo and 17 oxogenic steroids

Patient 8, aged 80 years

In this series synthetic oestrogens had little or no immediate effect on 17-oxosteroid excretion In four cases this fraction was depressed (No 5, 6, 7 and 8), and a minimum of 7 days treatment was required for the effect to become manifest In the fifth case (No 4), 17-oxosteroid excretion was unchanged over 102 weeks of treatment In only two of the five cases was there a return to normal levels

With the exception of Case No 4, 17-oxogenic steroid excretion was depressed by treatment the effect taking a week to become apparent This fall was maintained over a long period in three patients (No 5, 6 and 7) but in the remaining case (No 8), excretion had risen to the control level by 52 weeks

Oestrogen androgen ratios

The pre-treatment oestrogen levels for the 8 patients described were divided by the 17-oxosteroid levels and the ratios obtained compared with those obtained from the data for normal controls without prostatic cancer (Bulbrook, Franks and Greenwood, 1959) There was no significant difference between the mean ratios for the two groups (0 0011 and 0 0019 respectively) In both groups the daily oestrogen/17-oxosteroid ratio was fairly constant for a particular subject but there was a 20-fold variation between individual subjects

DISCUSSION

Changes in excretion after treatment

In a previous paper (Bulbrook, Franks and Greenwood, 1959) we have shown that the excretion of oestrogens, 17-oxosteroids and 17-oxogenic steroids in men with untreated prostatic cancer does not differ significantly from that found in patients of a similar age without clinical cancer However after treatment with synthetic oestrogens or by castration there is a temporary fall in the excretion of one or more of the three groups of hormones measured, followed by a rise to pre-treatment levels This biphasic response is shown most clearly by the oestrogens The time taken for these changes to occur varies from patient to patient Clinical improvement tends to precede marked decrease in hormone excretion, but the period of lowest hormone excretion was generally associated with retardation of tumour growth The increase in hormone excretion to control levels again generally preceded clinical signs of renewed tumour growth

When the results are considered in detail several further points arise There is little doubt about the biphasic response to oestrogen treatment or to castration , the fall and subsequent rise in excretion of at least one of the three groups of hormones is generally marked There are two other effects which are so slight that there must be some doubt as to their general occurrence The first of these is the slight and transient rise in 17-oxosteroids, 17-oxogenic steroids or oestrogens which sometimes occurs in the first two or three days after treatment with synthetic oestrogen is started In the 17-oxosteroid fraction this occurred in one patient, in the 17-oxogenic steroids and the oestrogens in 2 patients The subsequent fall in excretion was clearly manifest by about the seventh day of treatment

The second minor effect was seen in three cases (2, 5 and 6), one a castrate, the others treated with stilboestrol Hormone excretion fell, rose again to pre-treatment levels and then dropped again, over a period of several months Burt, Finney and Scott (1957) found a similar change in 3 patients treated by castration It is possible that this might have been found in other cases if specimens had been collected at shorter intervals of time These changes did not seem to be associated with changes in the clinical state and their significance is uncertain

The effect of castration on oestrogen excretion —In all three cases there was a rise in excretion in the immediate post-operative period which is thought to be an adrenal cortical reaction to operative stress A similar rise occurs after oophorectomy in the female (Bulbrook and Greenwood, 1957) Oestrogens are thought to be mainly produced by the testes in man but in two of the three cases (No 1 and 3) castration had virtually no immediate effect on oestrogen excretion, suggesting that this hormone was being produced in some other organ, presumably the adrenal Nevertheless, over the following three months, oestrogen excretion

slowly declined In Case 2 however oestrogen excretion fell to roughly one-third of the pre-operative level within 14 days and there was no evidence of a further fall at subsequent examinations

Variability of response to treatment—The pre-treatment hormone levels found in this small series differed widely from patient to patient Oestrogen excretion varied eight-fold between the patients, 17-oxosteroids three-fold and 17-oxogenic steroids four-fold Although the general pattern of response to treatment was similar, the time taken for the changes to occur and the absolute amounts of hormones excreted during treatment were equally varied This may be due to differences in response, from patient to patient, to a given dose of a synthetic oestrogen It is possible that the most effective dose varies as widely from patient to patient as insulin requirements in diabetics The variation in response to hormones may be genetically determined This is well known in experimental animals and it is likely that it also occurs in man

Not only does treatment elicit a variable response from patient to patient but in each, the three groups of hormones measured also vary independently The oestrogens, 17-oxosteroids and 17-oxogenic steroids do not necessarily rise and fall together and in some cases only one or two of the three groups of hormones show an effect It is probable that relative changes in excretion in the individual patient may be of greater significance than the absolute levels

Hormone excretion and clinical state—The similarity of oestrogen androgen ratio in patients with and without clinical cancer suggests that a disturbance of the endogenous production of these hormones is not associated with the development of clinical prostatic cancer However, there seems to be a rough correlation in this group of patients between the period of clinical improvement and lowered hormone excretion after treatment, particularly as far as the urinary oestrogens are concerned This association may be fortuitous and was not invariably found in another series of cases which had been treated with synthetic oestrogens for more than five years (Bulbrook, Franks and Greenwood, 1959) All the hormones measured in this study are produced in response to stimulation by trophic pituitary hormones If the primary endocrine disturbance associated with the growth of prostatic tumours is hypophyseal, the changes observed in steroid hormone excretion may not reflect the pituitary changes very accurately

Another possibility which must be considered is that there is a level, perhaps varying from patient to patient, above which tumour growth is maximally stimulated In this case the absolute amount of hormones produced would not necessarily be directly proportional to the growth activity

Possible sources of error

In any clinical investigation a number of possible sources of error must be recognised These include amongst others the accuracy of the original diagnosis, inadequate sample size and conscious or unconscious selection of cases In this investigation the clinical diagnosis has been confirmed microscopically in all cases but it must be realised that the microscopy gives no guide to the biological malignancy of the tumours (Franks, Fergusson and Murnaghan, 1958) In addition only a small number of patients has been studied but the findings are in general agreement with similar investigations In this group there has been some selection of patients, in that the severely ill patient, with urinary obstruction

or gross infection was unsuitable for investigation Similarly, patients with small " early " tumours were often treated as out-patients and not admitted to hospital The patients in this short-term group are therefore those with moderately severe disease but without gross urinary disturbance

Summary and conclusions

The urinary excretion of oestrogens, 17-oxosteroids and 17-oxogenic steroids was measured in 8 patients with prostatic cancer, before and at intervals during treatment by castration (3 cases) or synthetic oestrogens (5 cases) The excretion of these hormones is depressed by such treatment but in most cases there is a later rise to pretreatment levels This biphasic response is shown most clearly by the oestrogens Objective regression of tumour growth in these cases, is associated with depression of hormone excretion The later rise in excretion is generally followed by renewed tumour growth

There is a wide variation in the response to treatment from patient to patient and each of the three groups of hormones measured may vary independently

The changes measured may reflect changes in pituitary function This may be the basic endocrine factor involved

Our thanks are due to many people without whose help this investigation could not have been completed and in particular to Mr J D Fergusson, Institute of Urology, St Philip's and St Paul's Hospitals, under whose care many of these patients were admitted , Mr D Wallace, St Peter's Hospital and Mr G F Murnaghan, formerly Research Assistant, Institute of Urology

REFERENCES

BULBROOK, R D , FRANKS, L M AND GREENWOOD, F C —(1959) *Acta endocr , Copenhagen* (In the press)

Idem AND GREENWOOD, F C —(1957) *Brit med J* , 1, 662

BURT, F B , FINNEY, R P AND SCOTT, W W —(1957) *Cancer*, 10, 825

FRANKS, L M , FERGUSSON, J D AND MURNAGHAN, G F —(1958) *Brit J Cancer*, 12, 321

THE EFFECTS OF AGE ON THE STRUCTURE AND RESPONSE TO OESTROGENS AND TESTOSTERONE, OF THE MOUSE PROSTATE IN ORGAN CULTURES

L M FRANKS

From Imperial Cancer Research Fund Laboratories, Mill Hill, London, N W 7

Received for publication December 30, 1958

THE association between neoplasia and age is well seen in human prostatic cancer In this disease, although the incidence increases with age, there is often a marked decline in biological malignancy (Franks, 1956), but it is uncertain whether the change is due to an alteration in the tumour or to endocrine or other changes in the host, which are known to accompany ageing In addition the mechanism by which hormone-induced changes are produced in tumours or normal tissues in the intact animal remains obscure By using the organ culture technique in which tissue can be maintained in an organised form *in vitro* for several weeks, it is possible to study the local tissue (or tumour) factors in isolation, and to observe the direct effects of specific hormones There have been few reports however of studies of this type on adult tissues, except for those of Lasnitzki who, in a series of papers (Lasnitzki, 1951, 1954, 1955*a* and *b*), has described the effects of methylcholanthrene, oestrone, testosterone propionate and vitamin A on organ cultures of mouse prostate The present paper records the effects *in vitro* of oestrogens and testosterone on the ventral prostate of mice of different ages, and is preliminary to a more detailed study of the human and rodent prostate

MATERIAL AND METHODS

C57 mice were used in all except one series of experiments and killed by neck dislocation The ventral prostate was removed and each lobe of the paired gland was divided into two, or occasionally, if the lobe was very large, into four pieces Tissue from each mouse thus provided material for four or eight cultures, which were divided into four groups—one group grown on control medium and three on hormone-containing media The cultures were explanted on rayon (4 pieces on 1 cm squares) by Shaffer's (1956) modification of the standard watch glass technique (Fell and Robison, 1929) Each watch glass contained 2 pieces of rayon, i e , 8 cultures, on 1 5 ml of clotted medium, composed of 0 5 ml each of chicken plasma, chick embryo extract and human serum The hormones used were dissolved in the human serum, to which was also added sufficient chloromycetin to give a final concentration in the medium of approximately 0 03 mg /ml Cultures were incubated at 37° C and the medium changed every 3 or 4 days Eight cultures from each group were fixed after 1, 3, 7, 10 and 21 days in experiments 1, 2 and 3 The media used in each of these experiments were 1 Control medium 2 Control medium + oestrone 2 μg /ml 3 Control medium + oestrone 4 μg /ml 4 Control medium + testosterone propionate 50 μg /ml